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PLANKTON ANALYSIS TRAINING MANUAL

ENVIRONMENTAL PROTECTION AGENCY

MARCH 1975

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PLANKTON ANALYSIS

TRAINING MANUAL

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF WATER PROGRAMS

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PLANKTON ANALYSIS

This course is offered for professional personnel in the fields of water pollution control, limnology, and water supply. Primary emphasis is given to practice in the identification and enumeration of organisms which may be observed in the microscopic examination of water. Methods for the chemical and instrumental evaluation of plankton are compared with the results of microscopic examination in an extensive practical exercise. Problems of significance and control are also considered.

ENVIRONMENTAL PROTECTION AGENCY
Office of Water Program Operations
TRAINING PROGRAM

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LIMNOLOGY AND ECOLOGY OF PLANKTON

I INTRODUCTION

A Most Interference Organisms are Small.

B Small Organisms generally have Short Life Histories.

C Populations of Organisms with Short Life Histories may Fluctuate Rapidly in Response to Key Environmental Changes.

D Small Organisms are Relatively at the Mercy of the Elements

E The Following Discussion will Analyze the Nature of These Elements with Reference to the Response of Important Organisms.

II PHYSICAL FACTORS OF THE ENVIRONMENT

A Light is a Fundamental Source of Energy for Life and Heat.

1 Insolation is affected by geographical location and meteorological factors.

2 Light penetration in water is affected by angle of incidence (geographical), turbidity, and color. The proportion of light reflected depends on the angle of incidence, the temperature, color, and other qualities of the water. In general, as the depth increases arithmetically, the light tends to decrease geometrically. Blues, greens, and yellows tend to penetrate most deeply while ultra violet, violets, and orange-reds are most quickly absorbed. On the order of 90% of the total illumination which penetrates the surface film is absorbed in the first 10 meters of even the clearest water.

3 Turbidity may originate within or outside of a lake.

a That which comes in from outside (allochthonous) is predominately inert solids (tripton).

b That of internal origin (autochthonous) tends to be biological in nature.

B Heat and Temperature Phenomena are Important in Aquatic Ecology.

1 The total quantity of heat available to a body of water per year can be calculated and is known as the heat budget.

2 Heat is derived directly from insolation; also by transfer from air, internal friction, and other sources.

C Density Phenomena

1 Density and viscosity affect the floatation and locomotion of microorganisms.

a Pure fresh water achieves its maximum density at 4°C and its maximum viscosity at 0°C.

b The rate of change of density increases with the temperature.

2 Density stratification affects aquatic life and water uses.

a In summer, a mass of warm surface water, the epilimnion, is usually present and separated from a cool deeper mass, the hypolimnion, by a relatively thin layer known as the thermocline.

b Ice cover and annual spring and fall overturns are due to successive seasonal changes in the relative densities of the epilimnion and the hypolimnion, profoundly influenced by prevailing meteorological conditions.

c The sudden exchange of water masses having different chemical characteristics may have catastrophic effects on certain biota, may cause others to bloom.

d Silt laden waters may seek certain levels, depending on their own specific gravity in relation to existing layers already present.

e Saline waters will also stratify according to the relative densities of the various layers.

3 The viscosity of water is greater at lower temperatures.

a This is important not only in situations involving the control of flowing water as in a sand filter, but also since overcoming resistance to flow generates heat, it is significant in the heating of water by internal friction from wave and current action and many delay the establishment of anchor ice under critical conditions.

b It is easier for plankton to remain suspended in cold viscous (and also dense) water than in less viscous warm water. This is reflected in differences in the appearance of winter vs summer forms of life (also arctic vs tropical).

D Shore development, depth, inflow - outflow pattern, and topographic features affect the behavior of the water.

E Water movements that may affect organisms include such phenomena as waves, currents, tides, seiches, floods, and others.

1 Waves or rhythmic movement

a The best known are traveling waves. These are effective only against objects near the surface. They have little effect on the movement of large masses of water.

b Standing waves or seiches occur in all lakes but are seldom large enough to be observed. An "internal seich" is an oscillation in a density mass within a lake with no surface manifestation may cause considerable water movement.

2 Langmuire spirals (or Langmuire circulation are a relatively massive cylindrical motion imparted to surface waters under the influence of wind. The axes of the cylinders are parallel to the direction of the wind, and their depth and velocity depend on the depth of the water, the velocity and duration of the wind, and other factors. The net result is that adjacent cylinders tend to rotate in opposite directions like meshing cog wheels. Thus the water between two given spirals may be meeting and sinking, while that between spirals on either side will be meeting and rising. Water over the sinking areas tends to accumulate flotsam and jetsam on the surface in long conspicuous lines. Masses of microcrustacea attemping to stay near the surface may impart a reddish color to this water, and it is thus often referred to as the "red dance." The rising water on the other hand, having recently come from some depth, may (at least in the oceans or large lakes) have a bluish appearance, and is known as the "blue dance."

a This phenomenon is of considerable importance to those sampling for plankton (or even chemicals) near the surface when the wind is blowing. Grab samples from either dance might obviously differ considerably, and if a plankton tow is contemplated, it should be made across the wind in order that the net may pass through a succession of both dances.

b Langmuire spirals are not usually established until the wind has either been blowing for an extended period, or else is blowing rather hard. Their presence can be detected by the lines of foam and other floating material which coincide with the direction of the wind.

3 Currents

a Currents are arhythmic water movements which have had major study only in oceanography. They primarily are concerned with the translocation of water masses. They may be generated internally by virtue of density changes, or externally by wind or runoff.

b Turbulence phenomena or eddy currents are largely responsible for lateral mixing in a current. These are of far more importance in the economy of a body of water than mere laminar flow.

c Tides, or rather tidal currents, are reversible (or oscillatory) on a relatively long and predictable period. They are closely allied to seiches. For all practical purposes, they are restricted to oceanic (especially coastal) waters.

If there is no freshwater inflow involved, tidal currents are basically "in and out;" if a significant amount of freshwater is added to the system at a constant rate, the outflowing current will in general exceed the inflow by the amount of freshwater input.

There are typically two tidal cycles per lunar day (approximately 25 hours), but there is continuous gradation from this to only one cycle per (lunar) day in some places.

Estuarine plankton populations are extremely influenced by local tidal patterns.

d Flood waters range from torrential velocities which tear away and transport vast masses of substrate to quiet backwaters which may inundate normally dry land areas for extended periods of time. In the former case, planktonic life is flushed away completely; in the latter, a local plankton bloom may develop which may be of immediate significance, or which may serve as an inoculum for receding waters.

F Surface Tension and the Surface Film

1 The surface film is the habitat of the "neuston", a group of particular importance in water supplies.

2 Surface tension lowered by surfactants may eliminate the neuston. This can be a significant biological observation.

III DISSOLVED SUBSTANCES

A Carbon dioxide is released by plants and animals in respiration, but taken in by plants in photosynthesis.

B Oxygen is the biological complement of carbon dioxide, and necessary for all animal life.

C Nitrogen and phosphorus are fundamental nutrients for plant life.

1 Occur in great dilution, concentrated by plants.

2 The distribution of nitrogen compounds is generally correlated with the oxygen curve, especially in oceans.

D Iron, manganese, sulphur, and silicon are other minerals important to aquatic life which exhibit biological stratification.

E Many other minerals are present but their biological distribution in waters is less well known, fluorine, tin, and vanadium have recently been added to the "essential" list, and more may well follow.

F Dissolved organic matter is present in even the purest of lakes.

V BIOLOGICAL FACTORS

A Nutritional Classification of Organisms

1 Holophytic or independent organisms, like green plants, produce their own basic food elements from the physical environment.

2 Holozoic or dependent organisms, like animals, ingest and digest solid food particles of organic origin.

3 Saprophytic or carrion eating organisms, like many fungi and bacteria, digest and assimilate the dead bodies of other organisms or their products.

B The Prey-Predator Relationship is Simply one Organism Eating Another.

C Toxic and Hormonic Relationships

1 Some organisms such as certain blue green algae and some armored flagellages produce substances poisonous to others.

2 Antibiotic action in nature is not well understood but has been shown to play a very influential role in the economy of nature.

V BIOTIC COMMUNITIES (OR ECOSYSTEMS)

A A biotic community will be defined here as an assemblage of organisms living in a given ecological niche (as defined below). Producer (plant-like), consumer (animal-like) and reducer (bacteria and fungi) organisms are usually included. A source of energy (nutrient, food) must also be present. The essential concept in that each so-called community is a relatively independent entity. Actually this position is only tenable at any given instant, as individuals are constantly shifting from one community to another in response to stages in their life cycles, physical conditions, etc. The only one to be considered in detail here is the plankton.

B Plankton are the macroscopic and microscopic animals, plants, bacteria, etc. floating free in the open water. Many clog filters, cause tastes, odors, and other troubles in water supplies.

1 Those that pass through a plankton net (No. 25 silk bolting cloth or equivalent) or sand filter are often known as nannoplankton (they usually greatly exceed the "net" plankton in actual quantity).

2 Those less than four microns in length are sometimes called ultraplankton.

3 There are many ways in which plankton may be classified: taxonomic, ecological, industrial.

4 The concentration of plankton varies markedly in space and time.

a Depth, light, currents, and water quality profoundly affect plankton distribution.

b The relative abundance of plankton in the various seasons is generally:

1 spring, 2 fall, 3 summer, 4 winter

5 Marine plankton include many larger animal forms than are found in fresh waters.

C The benthic community is generally considered to be the macroscopic life living in or on the bottom.

D The periphyton community might be defined as the microscopic benthos, except that they are by no means confined to the bottom. Any surface, floating, or not, is usually covered by film of living organisms. There is frequent exchange between the periphyton and plankton communities.

E The nekton is the community of larger, free-swimming animals (fishes, shrimps, etc.), and so is dependent on the other communities for basic plant foods.

VI THE EVOLUTION OF WATERS

A The history of a body of water determines its present condition. Natural waters have evolved in the course of geologic time to what we know today.

B In the course of their evolution, streams in general pass through four general stages of development which may be called: birth, youth, maturity, and old age.

1 Establishment of birth. In an extant stream, this might be a "dry run" or headwater streambed, before it had eroded down to the level of ground water.

2 Youthful streams; when the stream bed is eroded below the ground water level, spring water enters and the stream becomes permanent.

3 Mature streams; have wide valleys, a developed flood plain, deeper, more turbid, and usually warmer water, sand, mud, silt, or clay bottom materials which shift with increase in flow.

4 In old age, streams have approached base level. During flood stage they scour their bed and deposit materials on the flood plain which may be very broad and flat. During normal flow the channel is refilled and many shifting bars are developed.

(Under the influence of man this pattern may be broken up, or temporarily interrupted. Thus as essentially "youthful" stream might take on some of the characteristics of a "mature" stream following soil erosion, organic enrichment, and increased surface runoff. Correction of these conditions might likewise be followed by at least a partial reversion to the "original" condition.)

C Lakes have a developmental history which somewhat parallels that of streams.

1 The method of formation greatly influences the character and subsequent history of lakes.

2 Maturing or natural eutrophication of lakes

a If not already present, shoal areas are developed through erosion of the shore by wave action and undertow.

b Currents produce bars across bays and thus cut off irregulars areas.

c Silt brought in by tributary streams settles out in the quiet lake water.

d Rooted aquatics grow on shoals and bars, and in doing so cut off bays and contribute to the filling of the lake.

e Dissolved carbonates and other materials are precipitated in the deeper portions of the lake in part through the action of plants.

f When filling is well advanced sphagnum mats extend outward from the shore. These mats are followed by sedges and grasses which finally convert the lake into a marsh.

3 Extinction of lakes. After lakes reach maturity their progress toward filling up is accelerated. They become extinct through:

a The downcutting of the outlet.

b Filling with detritus eroded from the shores or brought in by tributary streams.

c Filling by the accumulation of the remains of vegetable materials growing in the lake itself.

(Often two or three processes may act concurrently)

When man hastens the above process, it is often called "cultural eutrophication."

VI PRODUCTIVITY

A The biological resultant of all physical and chemical factors is the quantity of life that may actually be present. The ability to produce this "biomass" is often referred to as the "productivity" of a body of water. This is neither good nor bad per se. A water of low productivity is a "poor" water biologically, and also a relatively "pure" or "clean" water; hence desirable as a water supply. A productive water on the other hand may be a nuisance to man or highly desirable. Some of the factors which influence the productivity of waters are as follows:

B Factors affecting stream productivity. To be productive of plankton, a stream must provide adequate nutrients, light, a suitable temperature, and time for growth to take place.

1 Youthful streams, especially on rock or sand substrates are low in essential nutrients. Temperatures in mountainous regions are usually low, and due to the steep gradient, time for growth is short. Although ample light is available, growth of true plankton is thus greatly limited.

2 As the stream flows toward a more "mature" condition nutrients tend to accumulate, and gradient diminishes and so time of flow increases, temperature tends to increase, and plankton flourish.

Should a heavy load of inert silt develop on the other hand, the turbidity would reduce the light penetration and consequently the general plankton production would diminish.

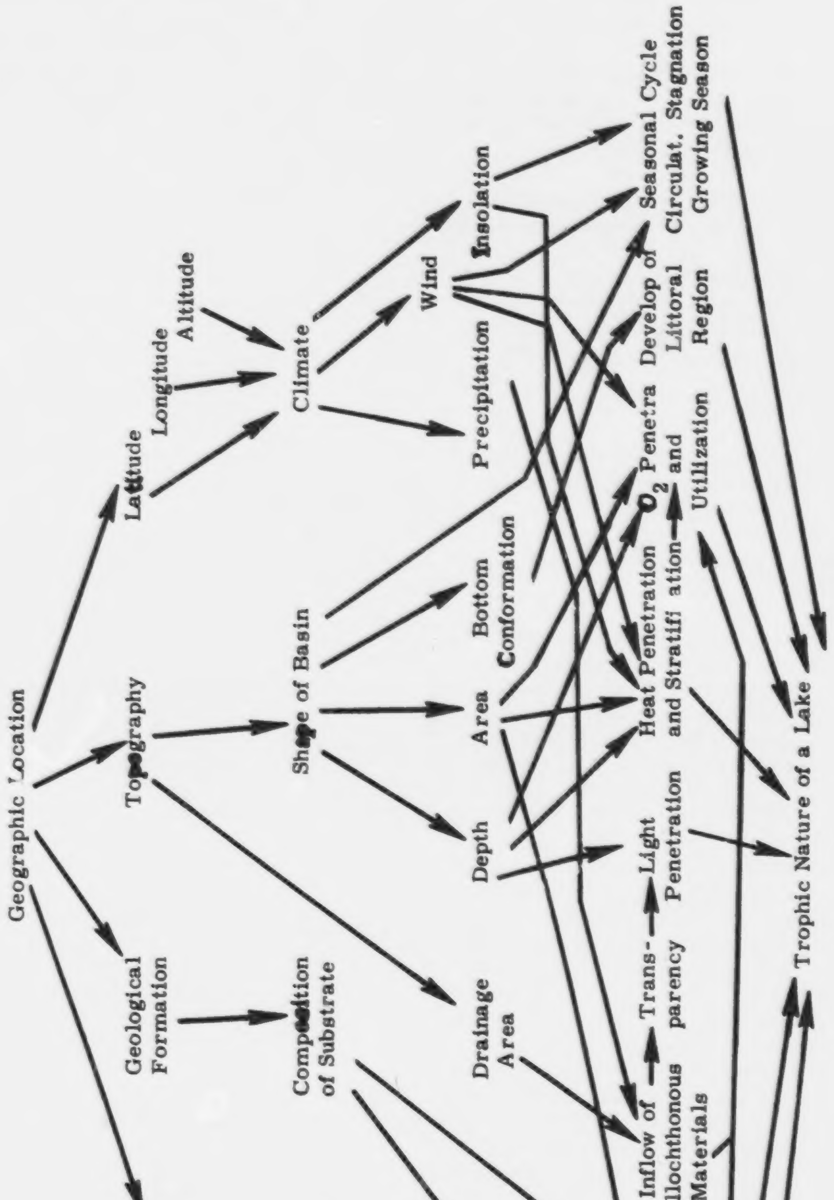
3 As the stream approaches base level (old age) and the time available for plankton growth increases, the balance between turbidity, nutrient levels, and temperature and other seasonal conditions, determines the overall productivity.

C Factors Affecting the Productivity of Lakes

1 The size, shape, and depth of the lake basin. Shallow water is more productive than deeper water since more light will reach the bottom to stimulate rooted plant growth. As a corollary, lakes with more shoreline, having more shallow water, are in general more productive. Broad shallow lakes and reservoirs have the greatest production potential (and hence should be avoided for water supplies).

2 Hard waters are generally more productive than soft waters as there are more plant nutrient minerals available. This is often

FACTORS AFFECTING PRODUCTIVITY


Geographic Location
Human Influence
Geological Formation
Topography
Latitude
Longitude
Altitude
Sewage Agriculture Mining
Composition of Substrate
Shape of Basin
Climate
Primary Nutritive Materials
Drainage Area
Depth
Area
Bottom Conformation
Precipitation
Wind
Insolation
Nature of Bottom Deposits
Inflow of Allochthonous Materials
Trans-parency
Light Penetration
Heat Penetration and Stratification
O2 Penetration and Utilization
Develop of Littoral Region
Seasonal Cycle Circulat. Stagnation Growing Season
Trophic Nature of a Lake

greatly influenced by the character of the soil and rocks in the watershed, and the quality and quantity of ground water entering the lake. In general, pH ranges of 6.8 to 8.2 appear to be most productive.

3 Turbidity reduces productivity as light penetration is reduced.

4 The presence or absence of thermal stratification with its semi-annual turnovers affect productivity by distributing nutrients throughout the water mass.

5 Climate, temperature, prevalance of ice and snow, are also important.

D Factors Affecting the Productivity of Reservoirs

1 The productivity of reservoirs is governed by much the same principles as that of lakes, with the difference that the water level is much more under the control of man. Fluctuations in water level can be used to deliberately increase of decrease productivity. This can be demonstrated by a comparison of the TVA reservoirs which practice a summer drawdown with some of those in the west where a winter drawdown is the rule.

2 The level at which water is removed from the reservoir is also important. The upper epilimnion may have a high plankton turbidity while lower down the plankton count may be less, but a taste and odor causer (such as Mallomonas) may be present. There may be two thermoclines, with a mass of muddy water flowing between a clear upper epilimnion and a clear hypolimnion. Other combinations ad infinitum may occur.

3 Reservoir discharges also profoundly affect the DO, temperature, and turbidity in the stream below a dam. Too much fluctuation in flow may permit sections of the stream to dry periodically.

VIII CLASSIFICATION OF LAKES AND RESERVOIRS

A The productivity of lakes and impoundments is such a conspicuous feature that it is often used as a means of classification.

1 Oligotrophic lakes are the geologically younger, less productive lakes, which are deep, have clear water, and usually support Salmonoid fishes.

2 Mesotropic lakes are generally intermediate between oligotrophic and eutrophic lakes. They are moderately productive, yet pleasant to be around.

3 Eutrophic lakes are more mature, more turbid, and richer. They are usually shallower. They are richer in dissolved solids; N, P, and Ca are abundant. Plankton is abundant and there is often a rich bottom fauna. Nuisance conditions often appear.

4 Dystrophic lakes - bog lakes - low in pH, water yellow to brown, dissolved solids; N, P, and Ca scanty but humic materials abundant; bottom fauna and plankton poor, and fish species are limited.

B Reservoirs may be classified as storage, or run of the river.

1 Storage reservoirs have a large volume in relation to their inflow.

2 Run of the river reservoirs have a large flow through in relation to their storage value.

C According to location, lakes and reservoirs may be classified as polar, temperate, or tropical. Differences in climatic and geographic conditions result in differences in their biology.

IX THE MANAGEMENT OR CONTROL OF ENVIRONMENTAL FACTORS

A Liebig's Law of the Minimum states that productivity is limited by the nutrient present in the least amoung at any given time relative to the assimilative capacity of the organism.

B Shelford's Law of Toleration:

Minimum Limit of toleration		Range of Optimum of factor	Maximum limit of toleration	
Absent	← Decreasing Abundance	Greatest abundance	→ Decreasing Abundance	Absent

C The artificial introduction of nutrients (sewage pollution or fertilizer) thus tends to eliminate existing limiting minimums for some species and create intolerable maximums for other species.

1 Known limiting minimums may sometimes be deliberately maintained.

2 As the total available energy supply is increased, productivity tends to increase.

3 As productivity increases, the whole character of the water may be changed from a meagerly productive clear water lake (oligotrophic) to a highly productive and usually turbid lake (eutrophic).

4 Eutrophication leads to treatment troubles.

D Control of eutrophication may be accomplished by carious means

1 Watershed management, adequate preparation of reservoir sites, and pollution control tend to maintain minimum limiting nutritional factors.

2 Shading out the energy of insolation by roofing or inert turbidity; suppresses photosynthesis.

3 Introduction of substances toxic to some fundamental part of the food chain (such as copper sulphate) tends to temporarily inhibit productivity.

X SUMMARY

A A body of water such as a lake represents an intricately balanced system in a state of dynamic equilibrium. Modification imposed at one point in the system automatically results in compensatory adjustments at associated points.

B The more thorough our knowledge of the entire system, the better we can judge where to impose control measures to achieve a desired result.

REFERENCES

1 Chamberlin, Thomas C., and Salisburg, Rollin P., Geology Vol. 1, "Geological Processes and Their Results", pp i-xix, and 1-654, Henry Holt and Company, New York, 1904.

2 Dorsey, N. Ernest. Properties of Ordinary Water - Substance. Reinhold Publ. Corp., New York. pp. 1-673. 1940.

3 Hutcheson, George E. A Treatise on Limnology. John Wiley Company. 1957.

4 Ruttner, Franz. Fundamentals of Limnology. University of Toronto Press. pp. 1-242. 1953.

5 Tarzwell, Clarence M. Experimental Evidence on the Value of Trout 1937 Stream Improvement in Michigan. American Fisheries Society Trans. 66:177-187. 1936.

6 US DHEW, PHS. Algae and Metropolitan Wastes, transactions of a seminar held April 27-29, 1960 at the Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio. No. SEC TR W61-3.

7 Ward and Whipple. Freshwater Biology (Introduction). John Wiley Company. 1918

8 Whittaker, R. H. Communities and Ecosystems. Macmillan, New York. 162 pp. 1970.

9 Zakdin, V. I. and Gerd, Sr. Fauna and Flora of the Lakes and Reservoirs of the USSR. Available from the Office of Technical Services, U. S. Dept. Commerce, Washington, DC.

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, DTTB, MDS, OWP, EPA, Cincinnati, OH 45268.

BIOLOGY OF ZOOPLANKTON COMMUNITIES

I CLASSIFICATION

A The planktonic community is composed of organisms that are relatively independent of the bottom to complete their life history. They inhabit the open water of lakes (pelagic zone). Some species have inactive or resting stages that lie on the bottom and carry the species through periods of stress; e.g., winter. A few burrow in the mud and enter the pelagic zone at night, but most live in the open water all the time that the species is present in an active form.

B Compared to the bottom fauna and flora, the plankton consists of relatively few kinds of organisms that are consistently and abundantly present. Two major categories are often called phytoplankton (plants) and zooplankton (animals), but this is based on an outmoded classification of living things. The modern tendency is to identify groupings according to their function in the ecosystem: Primary producers (photosynthetic organisms), consumers (zooplankton), and decomposers (heterotrophic bacteria and fungi).

C The primary difference then is nutritional; phytoplankton use inorganic nutrient elements and solar radiation. Zooplankton feed on particles, much of which can be phytoplankton cells, but can be bacteria or particles of dead organisms (detritus) originating in the plankton, the shore region, or the land surrounding the lake.

D The swimming powers of planktonic organisms is so limited that their horizontal distribution is determined mostly by movements of water. Some of the animals are able to swim fast enough that they can migrate vertically tens of meters each day, but they are capable of little horizontal navigation. At most, some species of crustaceans show a general avoidance of the shore areas during calm weather when the water is moving more slowly than the animals can swim. By definition, animals that are able to control their horizontal location are nekton, not plankton.

E In this presentation, a minimum of classification and taxonomy is used, but it should be realized that each group is typified by adaptations of structure on physiology that are related to the planktonic mode of existence. These adaptations are reflected in the classification.

II FRESHWATER ZOOPLANKTON

A The freshwater zooplankton is dominated by representatives of three groups of animals, two of them crustaceans: Copepoda, Cladocera, Rotifera. All have feeding mechanisms that permit a high degree of selectivity of food, and two can produce resting eggs that can withstand severe environmental conditions. In general the food of usual zooplankton populations ranges from bacteria and small algae to small animals.

B The Copepoda reproduce by a normal biparental process, and the females lay fertilized eggs in groups which are carried around in sacs until they hatch. The immature animals go through an elaborate development with many stages. The later stages have mouthparts that permit them to collect particles. In many cases, these are in the form of combs which remove small particles by a sort of filtration process. In others, they are modified to form grasping organs by which small animals or large algae are captured individually.

C The Cladocera (represented by Daphnia) reproduce much of the time by parthenogenesis, so that only females are present. Eggs are held by the mother in a brood chamber until the young are developed far enough to fend for themselves. The newborn animals look like miniature adults, and do not go through an elaborate series of developmental stages in the water as do the copepods. Daphnia has comb-formed filtering structures on some of its legs that act as filters.

D Under some environmental conditions the development of eggs is affected and males are produced. Fertilized eggs are produced that can resist freezing and drying, and these carry the population through unsatisfactory conditions.

E The Rotifera are small animals with a ciliated area on the head which creates currents used both for locomotion and for bringing food particles to the mouth. They too reproduce by parthenogenesis during much of the year, but production of males results in fertilized, resistant resting eggs. Most rotifers lay eggs one at a time and carry them until they hatch.

III ZOOPLANKTON POPULATION DYNAMICS

A In general, zooplankton populations are at a minimum in the cold seasons, although some species flourish in cold water. Species with similar food requirements seem to reproduce at different times of the year or are segregated in different layers of lakes.

B There is no single, simple measurement of activity for the zooplankton as a whole that can be used as an index of production as can the uptake of radioactive carbon for the phytoplankton. However, it is possible to find the rate of reproduction of the species that carry their eggs. The basis of the method is that the number of eggs in a sample taken at a given time represents the number of animals that will be added to the population during an interval that is equal to the length of time it takes the eggs to develop. Thus the potential growth rate of the populations can be determined. The actual growth rate, determined by successive samplings and counting, is less than the potential, and the difference is a measure of the death rate.

C Such measurements of birth and death rates permits a more penetrating analysis to be made of the causes of population change than if data were available for population size alone.

D Following is an indication of the major environmental factors in the control of zooplankton.

1 Temperature has an obvious effect in its general control of rates. In addition, the production and hatching of resting eggs may be affected.

2 Inorganic materials

Freshwater lakes vary in the content of dissolved solids according to the geological situation. The total salinity and proportion of different dissolved materials in water can affect the population. Some species are limited to soft water, others to saline waters, as the brine shrimp. The maximum population size developed may be related to salinity, but this is probably an indirect effect working through the abundance of nutrients and production of food.

3 Food supply

Very strong correlations have been found between reproduction and food supply as measured by abundance of phytoplankton. The rate of food supply can affect almost all aspects of population biology including rate of individual growth, time of maturity, rate of reproduction and length of life.

4 Apparently in freshwater, dissolved organic materials are of little nutritional significance, although some species can be kept if the concentration of dissolved material is high enough. Some species require definite vitamins in the food.

5 Effect of predation on populations

The kind, quantity and relative proportions of species strongly affected by grazing by vertebrate and invertebrate predators. The death rate of Daphnia is correlated with the abundance of a predator. Planktivorous fish (alewives) selectively feed on larger species, so a lake with alewives is dominated by the smaller species of crustaceans and large ones are scarce or absent.

6 Other aspects of zooplankton

Many species migrate vertically considerable distances each day. Typically, migrating species spend the daylight hours deep in the lake and rise toward the surface in late afternoon and early evening.

Some species go through a seasonal change of form (cyclomorphosis) which is not fully understood. It may have an effect in reducing predation.

REFERENCES

1 Baker, A. L. An Inexpensive Microsampler. Limnol. and Oceanogr. 15(5): 158-160. 1970.

2 Brooks, J. L. and Dodson, S. I. Predation, Body, Size, and Composition of Plankton. Science 150: 28-35. 1965.

3 Dodson, Stanley I. Complementary Feeding Niches Sustained by Size-Selective Predation, Limnology and Oceanography 15(1): 131-137.

4 Hutchinson, G. E. 1967. A Treatise on Limnology. Vol. II. Introduction to Lake Biology and the Limnoplankton. xi + 1115. John Wiley & Sons, Inc., New York.

5 Jossi, Jack W. Annotated Bibliography of Zooplankton Sampling Devices. USFWS. Spec. Sci.: Rep.-Fisheries. 609. 90 pp. 1970.

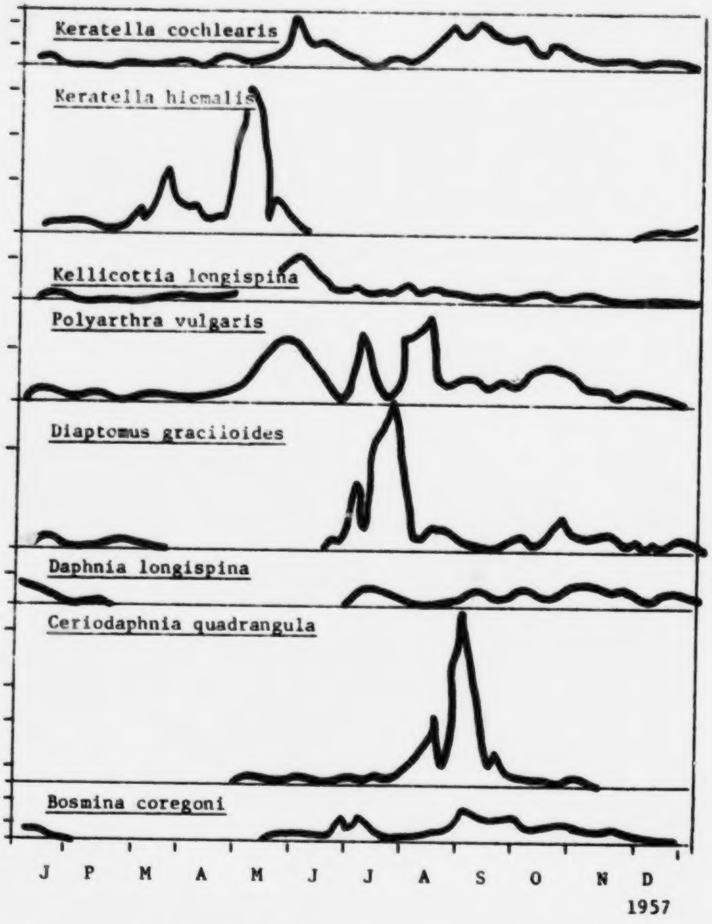
6 Likens, Gene E. and Gilbert, John J. Notes on Quantitative Sampling of Natural Populations of Planktonic Rotifers. Limnol. and Oceanogr. 15(5): 816-820.

7 Lund, J. W. G. 1965. The Ecology of the Freshwater Plankton. Biological Reviews, 40:231-293.

8 UNESCO. Zoolplankton Sampling. UNESCO Monogr. Oceanogr. Methodol. 2. 174 pp. 1968. (UNESCO. Place de Fortenoy, 75, Paris 7e France).

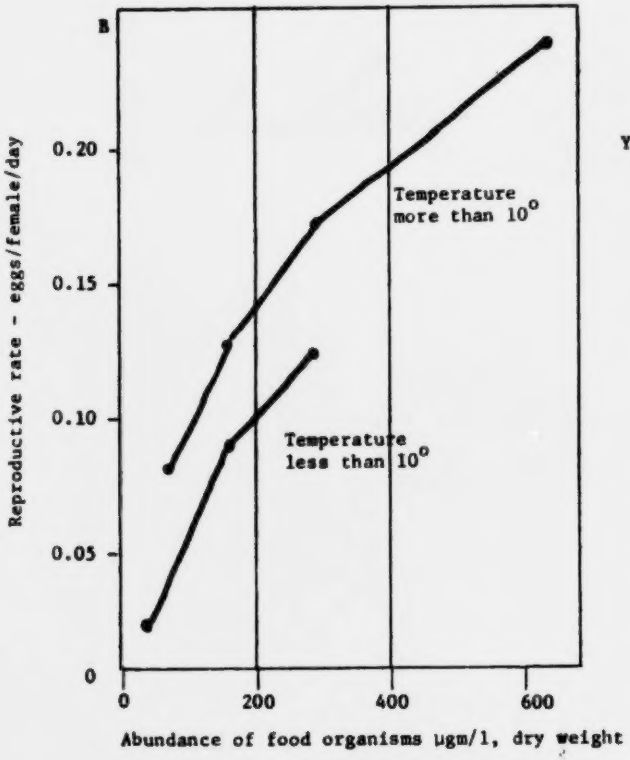
This outline was prepared by W. T. Edmondson, Professor of Zoology, University of Washington, Seattle, Washington.

FIGURE 1 SEASONAL CHANGES OF ZOOPLANKTON IN LAKE ERKEN, SWEDEN

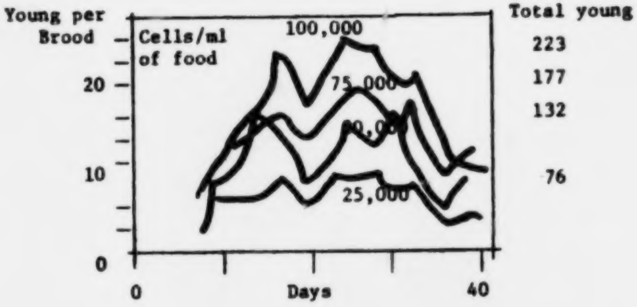


Each panel shows the abundance of a species of animal. Each mark on the vertical axis represents 10 individuals/liter. Nauwerck, A. 1963. Die Beziehungen zwischen Zooplankton und Phytoplankton im See Erken. Symbolae Botanicae Upsalienses, 17:1-163.

FIGURE 2 REPRODUCTIVE RATE OF ZOOPLANKTON AS A FUNCTION OF ABUNDANCE OF FOOD

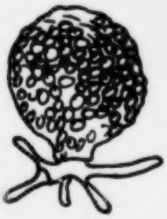


Mean rate of laying eggs by the planktonic rotifer Keratella cochlearis in natural populations as a function of abundance of food organisms and temperature. W. T. Edmonson. 1965. Reproductive rate of planktonic rotifers as related to food and temperature in nature. Ecol. Mmogr. 35: 61-111.



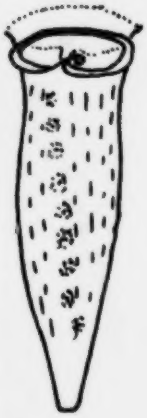
Number of young produced in each brood by Daphnia living in four different concentrations of food organisms, renewed daily. The total number produced during the life of a mother is shown by the numbers at the right. The Daphnia at the two lowest concentrations produced their first batch of eggs on the same day as the others, but the eggs degenerated, and the first viable eggs were released two days later. Richman, S. 1958. The transformation of energy by Daphnia pulex. Ecol. Monogr. 28: 273-291.

PROTOZOA

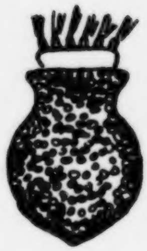


Difflugia

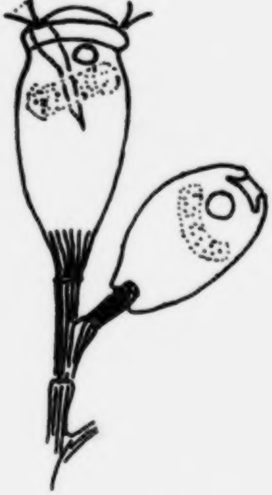
Amoebae



Stentor



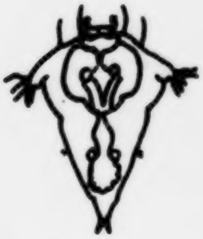
Codonella



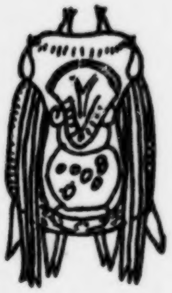
Epistylis

Ciliates

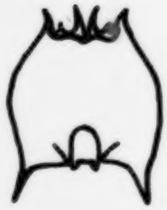
ROTIFERA



Synchaeta



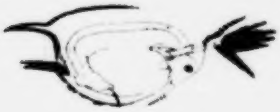
Polygarthra



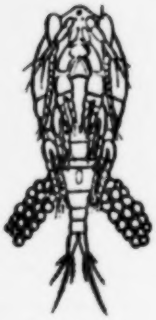
Brachionus

ARTHROPODA

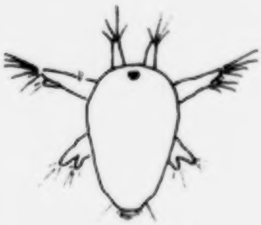
Crustacea



Cladocera



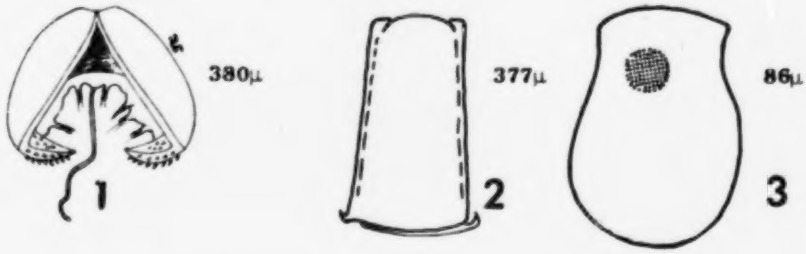
Copepoda



Nauplius larva of copepod

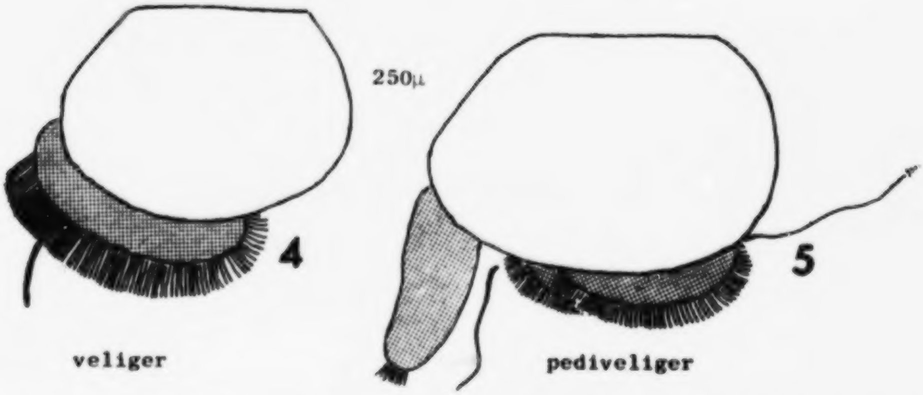
Insecta - Chaoborus

PLANKTONIC BIVALVE LARVAE



spined (fin attached) **simple** (gill attached)

Glochidia (Unionidae) Fish Parasites
(1-3)



veliger pediveliger

Veliger Larvae (Corbiculidae) Free Living Planktonic
(4-5)

Pediveliger attaches byssus lines)

OPTICS AND THE MICROSCOPE

I OPTICS

An understanding of elementary optics is essential to the proper use of the microscope. The microscopist will find that unusual problems in illumination and photomicrography can be handled much more effectively once the underlying ideas in physical optics are understood.

A Reflection

A good place to begin is with reflection at a surface or interface. Specular (or regular) reflection results when a beam of light leaves a surface at the same angle at which it reached it. This type of reflection occurs with highly polished smooth surfaces. It is stated more precisely as Snell's Law, *i.e.*, the angle of incidence, i, is equal to the angle of reflection, r (Figure 1). Diffuse (or scattered) reflection results when a beam of light strikes a rough or irregular surface and different portions of the incident light are reflected from the surface at different angles. The light reflected from a piece of white paper or a ground glass is an example of diffuse reflection.

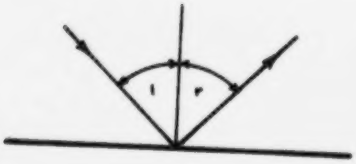


Figure 1

SPECULAR REFLECTION - SNELL'S LAW

Strictly speaking, of course, all reflected light, even diffuse, obeys Snell's Law. Diffuse reflected light is made up of many specularly reflected rays, each from a a tiny element of surface, and appears diffuse when the reflecting elements are very numerous and very small. The terms *diffuse* and *specular*, referring to reflection, describe not so much a difference in the nature of the reflection but rather a difference in the type of surface. A polished surface gives specular reflection; a rough surface gives diffuse reflection.

It is also important to note and remember that specularly reflected light tends to be strongly polarized in the plane of the reflecting surface. This is due to the fact that those rays whose vibration directions lie closest to the plane of the reflection surface are most strongly reflected. This effect is strongest when the angle of incidence is such that the tangent of the angle is equal to the refractive index of the reflecting surface. This particular angle of incidence is called the *Brewster angle*.

B Image Formation on Reflection

Considering reflection by mirrors, we find (Figure 2) that a plane mirror forms a virtual image behind the mirror, reversed right to left but of the same size as the object. The word *virtual* means that the image appears to be in a given plane but that a ground glass screen or a photographic film placed in that plane would show no image. The converse of a virtual image is a real image.

Spherical mirrors are either convex or concave with the surface of the mirror representing a portion of the surface of a sphere. The center of curvature is the center of the sphere, part of whose surface forms the mirror. The focus lies halfway between the center of curvature and the mirror surface.

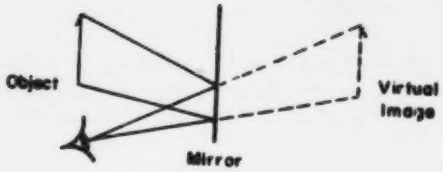


Figure 2

IMAGE FORMATION BY PLANE MIRROR

Construction of an image by a concave mirror follows from the two premises given below (Figure 3):

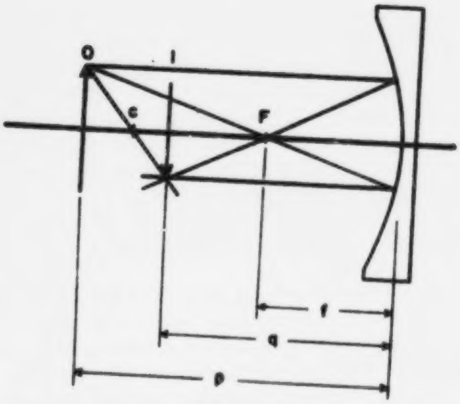


Figure 3

IMAGE FORMATION BY CONCAVE MIRROR

1 A ray of light parallel to the axis of the mirror must pass through the focus after reflection.

2 A ray of light which passes through the center of curvature must return along the same path.

A corollary of the first premise is:

3 A ray of light which passes through the focus is reflected parallel to the axis of the mirror.

The image from an object can be located using the familiar lens formula:

$$\frac{1}{p} + \frac{1}{q} \qquad \frac{1}{f}$$

where p = distance from the object to the mirror
q = distance from the image to the mirror
f = focal length

C Spherical Aberration

No spherical surface can be perfect in its image-forming ability. The most serious of the imperfections, spherical aberration, occurs in spherical mirrors of large aperture (Figure 4). The rays of light making up an image point from the outer zone of a spherical mirror do not pass through the same point as the more central rays. This type of aberration is reduced by blocking the outer zone rays from the image area or by using aspheric surfaces.

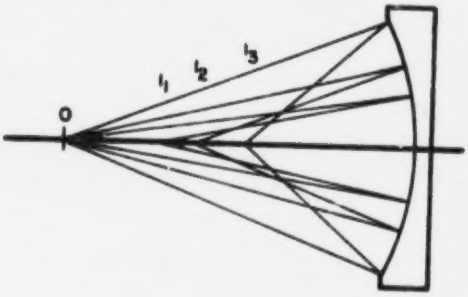


Figure 4

SPHERICAL ABERRATION BY SPHERICAL MIRROR

D Refraction of Light

Turning now to lenses rather than mirrors we find that the most important characteristic is refraction. Refraction refers to the change of direction and/or velocity of light as it passes from one medium to another. The ratio of the velocity in air (or more correctly in a vacuum) to the velocity in the medium is called the refractive index. Some typical values of refractive index measured with monochromatic light (sodium D line) are listed in Table 1.

Refraction causes an object immersed in a medium of higher refractive index than air to appear closer to the surface than it actually is (Figure 5). This effect may be used to determine the refractive index of a liquid with the microscope. A flat vial with a scratch on the bottom (inside) is placed on the stage of the microscope. The microscope is focused on the scratch and the fine adjustment micrometer reading is noted. A small amount of the unknown liquid is added; the scratch is again brought into focus and the new micrometer reading is taken. Finally, the microscope is refocused until the surface of the liquid appears in sharp focus. The micrometer reading is taken again and, with this information, the refractive index may be calculated from the simplified equation:

$$\text{refractive index} = \frac{\text{actual depth}}{\text{apparent depth}}$$

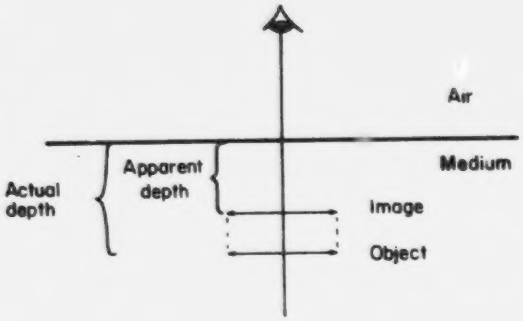


Figure 5

REFRACTION OF LIGHT AT INTERFACE

Table 1. REFRACTIVE INDICES OF COMMON MATERIALS MEASURED WITH SODIUM LIGHT

Vacuum	1.0000000	Crown glass	1.48 to 1.61
Air	1.0002918	Rock salt	1.5443
CO_2	1.0004498	Diamond	2.417
Water	1.3330	Lead sulfide	3.912

When the situation is reversed, and a ray of light from a medium of high refractive index passes through the interface of a medium of lower index, the ray is refracted until a critical angle is reached beyond which all of the light is reflected from the interface (Figure 6). This critical angle, C, has the following relationship to the refractive indices of the two media:

$$\sin C = \frac{n_2}{n_1}, \text{ where } n_2 < n_1.$$

When the second medium is air, the formula becomes:

$$\sin C = \frac{1}{n_1}.$$

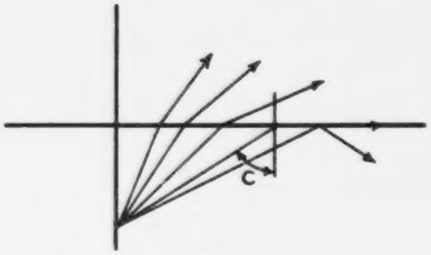


Figure 6

REFLECTION AT CRITICAL ANGLE

E Dispersion

Dispersion is another important property of transparent materials. This is the variation of refractive index with color (or wavelength) of light. When white light passes through a glass prism, the light rays are refracted by different amounts and separated into the colors of the spectrum. This spreading of light into its component colors is due to dispersion which, in turn, is due to the fact that the refractive index of transparent substances, liquids and solids, is lower for long wavelengths than for short wavelengths.

Because of dispersion, determination of the refractive index of a substance requires designation of the particular wavelength used. Light from a sodium lamp has a strong, closely spaced doublet with an average wavelength of 5893A, called the D line, which is commonly used as a reference wavelength. Table 2 illustrates the change of refractive index with wavelength for a few common substances.

Table 2. DISPERSION OF REFRACTIVE INDICES OF SEVERAL COMMON MATERIALS

	Refractive index		
	F line blue 4861A	D line (yellow) 5893A	C line (red) 6563A
Carbon disulfide	1.6523	1.6276	1.6182
Crown glass	1.5240	1.5172	1.5145
Flint glass	1.6391	1.6270	1.6221
Water	1.3372	1.3330	1.3312

The dispersion of a material can be defined quantitatively as:

$$\nu = \text{dispersion} = \frac{n\,(\text{yellow}) - 1}{n\,(\text{blue}) - n\,(\text{red})}$$

$$= \frac{n\,(593\text{m}\mu) - 1}{n\,(486\text{m}\mu) - n(656\text{m}\mu)}$$

where n is the refractive index of the material at the particular wavelength noted in the parentheses.

F Lenses

There are two classes of lenses, converging and diverging, called also convex and concave, respectively. The focal point of a converging lens is defined as the point at which a bundle of light rays parallel to the axis of the lens appears to converge after passing through the lens. The focal length of the lens is the distance from the lens to the focal point (Figure 7).

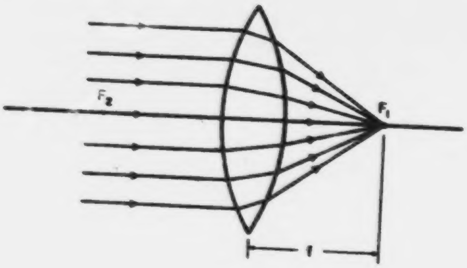


Figure 7

CONVERGENCE OF LIGHT AT FOCAL POINT

G Image Formation by Refraction

Image formation by lenses (Figure 8) follows rules analogous to those already given above for mirrors:

1 Light traveling parallel to the axis of the lens will be refracted so as to pass through the focus of the lens.

2 Light traveling through the geometrical center of the lens will be unrefracted.

The position of the image can be determined by remembering that a light ray passing through the focus, F, will be parallel to the axis of the lens on the opposite side of the lens and that a ray passing through the geometrical center of the lens will be unrefracted.

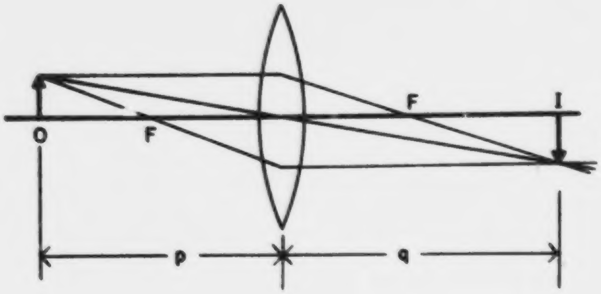


Figure 8

IMAGE FORMATION BY A CONVEX LENS

The magnification, M, of an image of an object produced by a lens is given by the relationship:

$$M = \frac{\text{image size}}{\text{object size}} = \frac{\text{image distance}}{\text{object distance}} = \frac{q}{p}$$

where q = distance from image to lens
and p = distance from object to lens.

H Aberrations of Lenses

Lenses have aberrations of several types which, unless corrected, cause loss of detail in the image. Spherical aberration appears in lenses with spherical surfaces. Reduction of spherical aberration can be accomplished by diaphragming the outer zones of the lens or by designing special aspherical surfaces in the lens system.

Chromatic aberration is a phenomenon caused by the variation of refractive index with wavelength (dispersion). Thus a lens receiving white light from an object will form a violet image closer to the lens and a red one farther away. Achromatic lenses are employed to minimize this effect. The lenses are combinations of two or more lens elements made up of materials having different dispersive powers. The use of monochromatic light is another obvious way of eliminating chromatic aberration.

Astigmatism is a third aberration of spherical lens systems It occurs when object points are not located on the optical axis of the lens and results in the formation of an indistinct image. The simplest remedy for astigmatism is to place the object close to the axis of the lens system.

I Interference Phenomena

Interference and diffraction are two phenomena which are due to the wave characteristics of light. The superposition of two light rays arriving simultaneously at a given point will give rise to interference effects, whereby the intensity at that point will vary from dark to bright depending on the phase differences between the two light rays.

The first requirement for interference is that the light must come from a single source. The light may be split into any number of paths but must originate from the same point (or coherent source). Two light waves from a coherent source arriving at a point in phase agreement will reinforce each other (Figure 9a). Two light waves from a coherent source arriving at a point in opposite phase will cancel each other (Figure 9b).

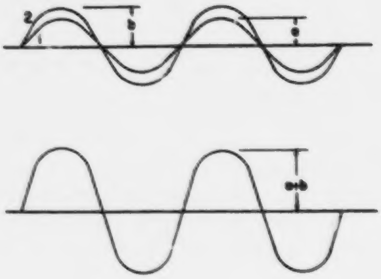


Figure 9a. Two light rays, 1 and 2, of the same frequency but different amplitudes, are in phase in the upper diagram. In the lower diagram, rays 1 and 2 interfere constructively to give a single wave of the same frequency and with an amplitude equal to the summation of the two former waves.

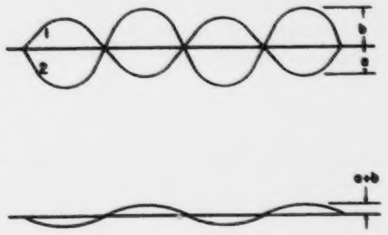


Figure 9b. Rays 1 and 2 are now 180° out of phase and interfere destructively. The resultant, in the bottom diagram, is of the same frequency but is of reduced amplitude (a is negative and is subtracted from b).

The reflection of a monochromatic light beam by a thin film results in two beams, one reflected from the top surface and one from the bottom surface. The distance traveled by the latter beam in excess of the first is twice the thickness of the film and its equivalent air path is:

$$2nt$$

where n is the refractive index and t is the thickness of the film.

The second beam, however, upon reflection at the bottom surface, undergoes a half wavelength shift and now the total retardation of the second beam with respect to the first is given as:

$$\text{retardation} = 2nt + \frac{\lambda}{2}$$

where λ is the wavelength of the light beam.

When retardation is exactly an odd number of half wavelengths, destructive interference takes place resulting in darkness. When it is zero or an even number of half wavelengths, constructive interference results in brightness (Figure 10).

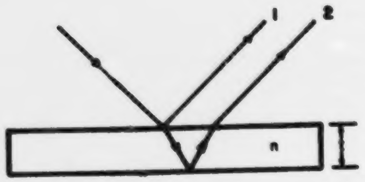


Figure 10

INTERFERENCE IN A THIN FILM

A simple interferometer can be made by partially silvering a microscope slide and cover slip. A preparation between the two partially silvered surfaces will show interference fringes when viewed with monochromatic light, either transmitted or by vertical illuminator. The fringes will be close together with a wedge-shaped preparation and will reflect refractive index differences due to temperature variations, concentration differences, different solid phases, etc. The method has been used to measure quantitatively the concentration of solute around a growing crystal(1) (Figure 11).

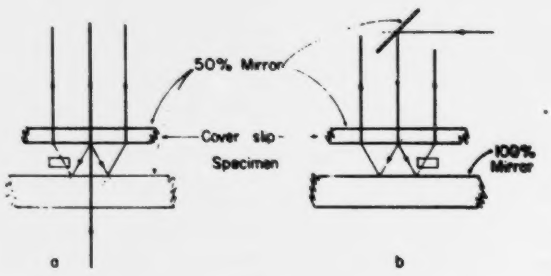


Figure 11
MICROSCOPICAL METHOD OF VIEWING INTERFERENCE IMAGES

a Examination is by transmitted light. Light ray undergoes multiple reflections and produces dark and light fringes in the field. A specimen introduces a phase shift and changes the fringe pattern.

b Illumination is from the top. The principle is the same but fringes show greater contrast.

Each dark band represents an equivalent air thickness of an odd number of half wavelengths. Conversely, each bright band is the result of an even number of half wavelengths.

With interference illumination, the effect of a transparent object of different refractive index than the medium in the microscope field is:

1 a change of light intensity of the object if the background is uniformly illuminated (parallel cover slip), or

2 a shift of the interference bands within the object if the background consists of bands (tilted cover slip).

The relationship of refractive indices of the surrounding medium and the object is as follows:

$$n_s = n_m\left(1 + \frac{\theta\lambda}{360t}\right)$$

where n_s = refractive index of the specimen
n_m = refractive index of the surrounding medium
θ = phase shift of the two beams, degrees
λ = wavelength of the light
t = thickness of the specimen.

J Diffraction

In geometrical optics, it is assumed that light travels in straight lines. This is not always true. We note that a beam passing through a slit toward a screen creates a bright band wider than the slit with alternate bright and dark bands appearing on either side of the central bright band, decreasing in intensity as a function of the distance from the center. Diffraction describes this phenomenon and, as one of its practical consequences, limits the lens in its ability to reproduce an image. For example, the image of a pin point of light produced by a lens is not a pin point but is revealed to be a somewhat larger patch of light surrounded by dark and bright rings. The diameter, d, of this diffraction disc (to the first dark ring) is given as:

$$d = \frac{2.44\, f\lambda}{D}$$

where f is the focal length of the lens, λ the wavelength, and D the diameter of the lens.

It is seen that in order to maintain a small diffraction disc at a given wavelength, the diameter of the lens should be as large as possible with respect to the focal length. It should be noted, also, that a shorter wavelength produces a smaller disc.

If two pin points of light are to be distinguished in an image, their diffraction discs must not overlap more than one half their diameters. The ability to distinguish such image points is called resolving power and is expressed as one half of the preceding expression:

$$\text{resolving power} = \frac{1.22\, f\lambda}{D}.$$

II THE COMPOUND MICROSCOPE

The compound microscope is an extension in principle of the simple magnifying glass; hence it is essential to understand fully the properties of this simple lens system.

A Image Formation by the Simple Magnifier

The apparent size of an object is determined by the angle that is formed at the eye by the extreme rays of the object. By bringing the object closer to the eye, that angle (called the visual angle) is increased. This also increases the apparent size. However a limit of accommodation of the eye is reached, at which distance the eye can no longer focus. This limiting distance is about 10 inches or 25 centimeters. It is at this distance that the magnification of an object observed by the unaided eye is said to be unity. The eye can, of course, be focused at shorter distances but not usually in a relaxed condition.

A positive, or converging, lens can be used to permit placing an object closer than 10 inches to the eye (Figure 12). By this means the visual angle of the object is increased (as is its apparent size) while the image of

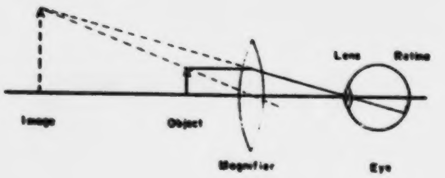


Figure 12

VIRTUAL IMAGE FORMATION BY CONVEX LENS

the object appears to be 10 inches from the eye, where it is best accommodated.

B Magnification by a Single Lens System

The magnification, M, of a simple magnifying glass is given by:

$$M = \frac{25}{f} + 1$$

where f = focal length of the lens in centimeters.

Theoretically the magnification can be increased with shorter focal length lenses. However such lenses require placing the eye very close to the lens surface and have much image distortion and other optical aberrations. The practical limit for a simple magnifying glass is about 20X.

In order to go to magnifications higher than 20X, the compound microscope is required. Two lens systems are used to form an enlarged image of an object (Figure 13). This is accomplished in two steps, the first by a lens called the objective and the second by a lens known as the eyepiece (or ocular).

C The Objective

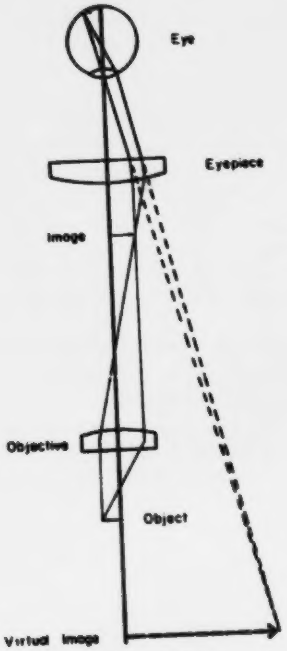


Figure 13

IMAGE FORMATION IN COMPOUND MICROSCOPE

The objective is the lens (or lens system) closest to the object. Its function is to reproduce an enlarged image of the object in the body tube of the microscope. Objectives are available in various focal lengths to give different magnifications (Table 3). The magnification is calculated from the focal length by dividing the latter into the tube length, usually 160 mm.

The numerical aperture (N. A.) is a measure of the ability of an objective to resolve detail. This is more fully discussed in the next section. The working distance is in the free space between the objective and the cover slip and varies slightly for objectives of the same focal length depending upon the degree of correction and the manufacturer.

There are three basic classifications of objectives: achromats, fluorites and apochromats, listed in the order of their complexity. The achromats are good for routine work while the fluorites and apochromats offer additional optical corrections to compensate for spherical, chromatic and other aberrations.

Table 3. NOMINAL CHARACTERISTICS OF USUAL MICROSCOPE OBJECTIVES

Nominal focal length mm	Nominal magnif.	N.A.	Working distance mm	Depth focus μ	Diam. of field mm.	Resolving power, white light, μ	Maximum useful magnif.	Eyepiece for max. useful magnif.
56	2.5X	0.08	40	50	8.5	4.4	80X	30X
32	5	0.10	25	16	5	3.9	90X	20X
16	10	0.25	7	8	2	1.4	250X	25X
8	20	0.50	1.3	2	1	0.7	500X	25X
4	43	0.66	0.7	1	0.5	0.4	660X	15X
4	45	0.85	0.5	1	0.4	0.35	850X	20X
1.8	90	1.30	0.2	0.4	0.2	0.21	1250X	12X

Another system of objectives employs reflecting surfaces in the shape of concave and convex mirrors. Reflection optics, because they have no refracting elements, do not suffer from chromatic aberrations as ordinary refraction objectives do. Based entirely on reflection, reflecting objectives are extremely useful in the infrared and ultraviolet regions of the spectrum. They also have a much longer working distance than the refracting objectives.

The body tube of the microscope supports the objective at the bottom (over the object) and the eyepiece at the top. The tube length is maintained at 160 mm except for Leitz instruments, which have a 170-mm tube length.

The objective support may be of two kinds, an objective clutch changer or a rotating nosepiece:

1 The objective clutch changer ("quick-change" holder) permits the mounting of only one objective at a time on the microscope. It has a centering arrangement, so that each objective need be centered only once with respect to the stage rotation. The changing of objectives with this system is somewhat awkward compared with the rotating nosepiece.

2 The revolving nosepiece allows mounting three or four objectives on the microscope at one time (there are some nosepieces that accept five and even six objectives). In this system, the objectives are usually noncenterable and the stage is centerable. Several manufacturers provide centerable objective mounts so that each objective on the nosepiece need be centered only once to the fixed rotating stage. The insides of objectives are better protected from dust by the rotating nosepiece. This, as well as the inconvenience of the so-called "quick-change" objective holder, makes it worthwhile to have one's microscope fitted with rotating nosepiece.

D The Ocular

The eyepiece, or ocular, is necessary in the second step of the magnification process. The eyepiece functions as a simple magnifier viewing the image formed by the objective.

There are three classes of eyepieces in common use: huyghenian, compensating and flat-field. The huyghenian (or huyghens) eyepiece is designed to be used with achromats while the compensating type is used with fluorite and apochromatic objectives. Flat-field eyepieces, as the name implies, are employed in photomicrography or projection and can be used with most objectives. It is best to follow the recommendations of the manufacturer as to the proper combination of objective and eyepiece.

The usual magnifications available in oculars run from about 6X up to 25 or 30X. The 6X is generally too low to be of any real value while the 25 and 30X oculars have slightly poorer imagery than medium powers and have a very low eyepoint. The most useful eyepieces lie in the 10 to 20X magnification range.

E Magnification of the Microscope

The total magnification of the objective-eyepiece combination is simply the product of the two individual magnifications. A convenient working rule to assist in the proper choice of eyepieces states that the maximum useful magnification (MUM) for the microscope is 1,000 times the numerical aperture (N. A.) of the objective. The MUM is related to resolving power in that magnification in excess of MUM gives little or no additional resolving power and results in what is termed empty magnification. Table 4 shows the results of such combinations and a comparison with the 1000X N. A. rule. The underlined figure shows the magnification nearest to the MUM and the eyepiece required with each objective to achieve the MUM. From this table it is apparent that only higher power eyepieces can give full use of the resolving power of the objectives. It is obvious that a 10X, or even a 15X, eyepiece gives insufficient magnification for the eye to see detail actually resolved by the objective.

F Focusing the Microscope

The coarse adjustment is used to roughly position the body tube (in some newer microscopes, the stage) to bring the image into focus. The fine adjustment is used after the coarse adjustment to bring the image into perfect focus and to maintain the focus as the slide is moved across the stage. Most microscope objectives are parfocal so that once they are focused any other objective can be swung into position without the necessity of refocusing except with the fine adjustment.

The student of the microscope should first learn to focus in the following fashion, to prevent damage to a specimen or objective:

1 Raise the body tube and place the specimen on the stage.

2 Never focus the body tube down (or the stage up) while observing the field through the eyepiece.

3 Lower the body tube (or raise the stage) with the coarse adjustment while carefully observing the space between the

Table 4. MICROSCOPE MAGNIFICATION CALCULATED FOR VARIOUS OBJECTIVE-EYEPIECE COMBINATIONS

Objective		Eyepiece					
Focal length	Magnification	5X	10X	15X	20X	25X	MUM[a] (1000 NA)
56mm	3X	15X	30X	45X	60X	75X	80X
32	5	25X	50X	75X	100X	125X	100X
16	10	50X	100X	150X	200X	250X	250X
8	20	100X	200X	300X	400X	500X	500X
4	40	200X	400X	600X	800X	1000X	660X
1.8	90	450X	900X	1350X	1800X	2250X	1250X

[a] MUM = maximum useful magnification

objective and slide and permitting the two to come close together without touching.

4 Looking through the microscope and turning the fine adjustment in such a way as to move the objective away from the specimen, bring the image into sharp focus.

The fine adjustment is usually calibrated in one- or two-micron steps to indicate the vertical movement of the body tube. This feature is useful in making depth measurements but should not be relied upon for accuracy.

G The Substage Condenser

The substage holds the condenser and polarizer. It can usually be focused in a vertical direction so that the condenser can be brought into the correct position with respect to the specimen for proper illumination. In some models, the condenser is centerable so that it may be set exactly in the axis of rotation of the stage; otherwise it will have been precentered at the factory and should be permanent.

H The Microscope Stage

The stage of the microscope supports the specimen between the condenser and objective, and may offer a mechanical stage as an attachment to provide a means of moving the slide methodically during observation. The polarizing microscope is fitted with a circular rotating stage to which a mechanical stage may be added. The rotating stage, which is used for object orientation to observe optical effects, will have centering screws if the objectives are not centerable, or vice versa. It is undesirable to have both objectives and stage centerable as this does not provide a fixed reference axis.

I The Polarizing Elements

A polarizer is fitted to the condenser of all polarizing microscopes. In routine instruments, the polarizer is fixed with its vibration direction oriented north-south (east-west for most European instruments) while in research microscopes, the polarizer can be rotated. Modern instruments have polarizing filters (such as Polaroid) replacing the older calcite prisms. Polarizing filters are preferred because they:

1 are low-cost;

2 require no maintenance;

3 permit use of the full condenser aperture.

An analyzer, of the same construction as the polarizer, is fitted in the body tube of the microscope on a slider so that it may be easily removed from the optical path. It is oriented with its plane of vibration perpendicular to the corresponding direction of the polarizer.

J The Bertrand Lens

The Bertrand lens is usually found only on the polarizing microscope although some manufacturers are beginning to include it on phase microscopes. It is located in the body tube above the analyzer on a slider (or pivot) to permit quick removal from the optical path. The Bertrand lens is used to observe the back focal plane of the objective. It is convenient for checking quickly the type and quality of illumination, for observing interference figures of crystals, for adjusting the phase annuli in phase microscopy and for adjusting the annular and central stops in dispersion staining.

K The Compensator Slot

The compensator slot receives compensators (quarter-wave, first-order red and quartz-wedge) for observation of the optical properties of crystalline materials. It is usually placed at the lower end of the body tube just above the objective mount, and is oriented 45° from the vibration directions of the polarizer and analyzer.

L The Stereoscopic Microscope

The stereoscopic microscope, also called the binocular, wide-field, dissecting or

Greenough binocular microscope, is in reality a combination of two separate compound microscopes. The two microscopes, usually mounted in one body, have their optical axes inclined from the vertical by about 7° and from each other by twice this angle. When an object is placed on the stage of a stereoscopic microscope, the optical systems view it from slightly different angles, presenting a stereoscopic pair of images to the eyes, which fuse the two into a single three-dimensional image.

The objectives are supplied in pairs, either as separate units to be mounted on the microscope or, as in the new instruments, built into a rotating drum. Bausch and Lomb was the first manufacturer to have a zoom lens system which gives a continuous change in magnification over the full range. Objectives for the stereomicroscope run from about 0.4X to 12X, well below the magnification range of objectives available for single-objective microscopes.

The eyepieces supplied with stereoscopic microscopes run from 10 to 25X and have wider fields than their counterparts in the single-objective microscopes.

Because of mechanical limitations, the stereomicroscope is limited to about 200X magnification and usually does not permit more than about 120X. It is most useful at relatively low powers in observing shape and surface texture, relegating the study of greater detail to the monocular microscope. The stereomicroscope is also helpful in manipulating small samples, separating ingredients of mixtures, preparing specimens for detailed study at higher magnifications and performing various mechanical operations under microscopical observation, e. g. micromanipulation.

III ILLUMINATION AND RESOLVING POWER

Good resolving power and optimum specimen contrast are prerequisites for good microscopy. Assuming the availability of suitable optics (ocular, objectives and substage condenser) it is still of paramount importance to use proper illumination. The requirement for a good illumination system for the microscope is to have uniform intensity of illumination over the entire field of view with independent control of intensity and of the angular aperture of the illuminating cone.

A Basic Types of Illumination

There are three types of illumination (Table 5) used generally:

1 Critical. This is used when high levels of illumination intensity are necessary for oil immersion, darkfield, fluorescence, low birefringence or photomicrographic studies. Since the lamp filament is imaged in the plane of the specimen, a ribbon filament or arc lamp is required. The lamp must be focusable and have an iris diaphragm; the position of the filament must also be adjustable in all directions.

2 Köhler. Also useful for intense illumination, Köhler illumination may be obtained with any lamp not fitted with a ground glass. The illuminator must, however, be focusable, it must have an adjustable field diaphragm (iris) and the lamp filament position must be adjustable in all directions.

3 "Poor man's". So-called because a low-priced illuminator may be used, this method gives illumination of high quality although of lower intensity because of the presence of a ground glass in the system. No adjustments are necessary on the illuminator or lamp filament although an adjustable diaphragm on the illuminator is helpful.

All three types of illumination require that the microscope substage condenser focus the image of the illuminator aperture in the plane of the specimen. In each case, then, the lamp iris acts as a field diaphragm and should be closed to just illuminate the field of view. The differences in these three types of illumination lie in the adjustment of the lamp condensing lens. With poor man's illumination there is no lamp condenser, hence no adjustment. The lamp should be placed close to the microscope so that

Table 5. COMPARISON OF CRITICAL, KOHLER AND POOR MAN'S ILLUMINATION

	Critical	Köhler	Poor man's
Lamp filament	ribbon filament	any type	any type
Lamp condensing lens	required	required	none
Lamp iris	required	required	useful
Ground glass at lamp	none	none	present
Image of light source	in object plane	at substage iris	none
Image of field iris	near object plane	in object plane	near object plane
Image of substage iris	back focal plane of objective	back focal plane of objective	back focal plane of objective

the entire field of view is always illuminated. If the surface structure of the ground glass becomes apparent in the field of view the substage condenser is very slightly defocused.

Critical Illumination

With critical illumination the lamp condenser is focused to give parallel rays; focusing the lamp filament on a far wall is sufficient. Aimed, then, at the substage mirror, the substage condenser will focus the lamp filament in the object plane. The substage condenser iris will now be found imaged in the back focal plane of the objective; it serves as a control over convergence of the illumination. Although the substage iris also affects the light intensity over the field of view it should most decidedly not be used for this purpose. The intensity of illumination may be varied by the use of neutral density filters and, unless color photomicrography is anticipated, by the use of variable voltage on the lamp filament.

Köhler illumination (Figure 14) differs from critical illumination in the use of the lamp condenser. With critical illumination the lamp condenser focuses the lamp filament at infinity; with Kohler illumination the lamp filament is focused in the plane of the substage condenser iris (also coincident with the anterior focal plane of the substage condenser). The functions of the lamp condenser iris and the substage condenser iris in controlling, respectively, the area of the illuminated field of view and the angular aperture of the illuminating cone are precisely alike for all three types of illumination.

Critical illumination is seldom used because it requires a special lamp filament and because, when used, it shows no advantage over well-adjusted Köhler illumination.

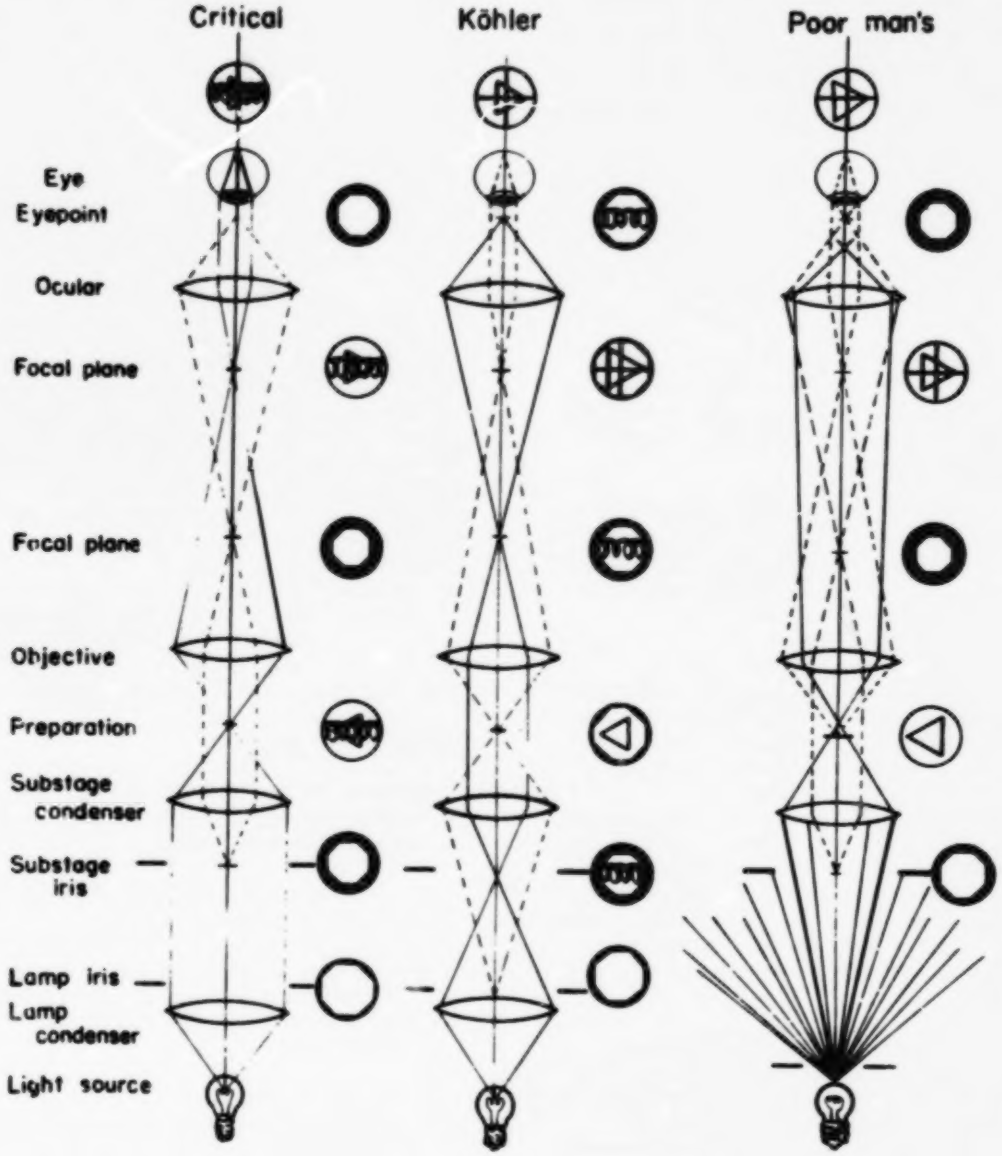
Köhler Illumination

To arrange the microscope and illuminator for Köhler illumination it is well to proceed through the following steps:

a Remove the diffusers and filters from the lamp.

b Turn the lamp on and aim at a convenient wall or vertical screen about 19 inches away. Open the lamp diaphragm.

c By moving the lamp condenser, focus a sharp image of the filament. It should be of such a size as to fill, not necessarily evenly, the microscope


Critical
Köhler
Poor man's
Eye
Eyepoint
Ocular
Focal plane
Focal plane
Objective
Preparation
Substage condenser
Substage iris
Lamp iris
Lamp condenser
Light source

substage condenser opening. If it does not, move the lamp away from the wall to enlarge the filament image, refocus.

d Turn the lamp and aim it at the microscope mirror so as to maintain the same 18 inches (or adjusted lamp distance).

e Place a specimen on the microscope stage and focus sharply with a 16-mm (10X) objective. Open fully the aperture diaphragm in the substage condenser. If the light is too bright, temporarily place a neutral density filter or a diffuser in the lamp.

f Close the lamp diaphragm, or field diaphragm, to about a 1-cm opening. Rack the microscope substage condenser up and down to focus the field diaphragm sharply in the same plane as the specimen.

g Adjust the mirror to center the field diaphragm in the field of view.

h Remove the 16-mm objective and replace with a 4-mm objective. Move the specimen so that a clear area is under observation. Place the Bertrand lens in the optical path, or remove the eyepiece and insert an auxiliary telescope (sold with phase contrast accessories) in its place, or remove the eyepiece and observe the back aperture of the objective directly. Remove any ground glass diffusers from the lamp. Now observe the lamp filament through the microscope.

i If the filament does not appear to be centered, swing the lamp housing in a horizontal arc whose center is at the field diaphragm. The purpose is to maintain the field diaphragm on the lamp in its centered position. If a vertical movement of the filament is required, loosen the bulb base and slide it up or down. If the base is fixed, tilt the lamp housing in a vertical arc with the field diaphragm as the center of movement (again endeavoring to keep the lamp diaphragm in the centered position).

If you have mastered this step, you have accomplished the most difficult portion. (Better microscope lamps have adjustments to move the bulb independently of the lamp housing to simplify this step.)

j Put the specimen in place, replace the eyepiece and the desired objective and refocus.

k Open or close the field diaphragm until it just disappears from the field.

l Observe the back aperture of the objective, preferably with the Bertrand lens or the auxiliary telescope, and close the aperture diaphragm on the substage condenser until it is about four-fifths the diameter of the back aperture. This is the best position for the aperture diaphragm, a position which minimizes glare and maximizes the resolving power. It is instructive to vary the aperture diaphragm and observe the image critically during the manipulation.

m If the illumination is too great, insert an appropriate neutral density filter between the illuminator and the condenser. Do not use the condenser aperture diaphragm or the lamp field diaphragm to control the intensity of illumination.

Poor Man's Illumination

Both critical and Köhler illumination require expensive illuminators with adjustable focus, lamp iris and adjustable lamp mounts. Poor man's illumination requires a cheap illuminator although an expensive illuminator may be used if its expensive features are negated by inserting a ground glass diffuser or by using a frosted bulb. Admittedly an iris diaphragm on the lamp would be a help though it is not necessary.

a The illuminator must have a frosted bulb or a ground glass diffuser.

It should be possible to direct it in the general direction of the substage mirror, very close thereto or in place thereof.

b Focus on any preparation after tilting the mirror to illuminate the field.

c Remove the top lens of the condenser and, by racking the condenser up or, more often, down, bring into focus (in the same plane as the specimen) a finger, pencil or other object placed in the same general region as the ground glass diffuser on the lamp. The glass surface itself can then be focused in the plane of the specimen.

d Ideally the ground glass surface will just fill the field of view when centered by the substage mirror; adjustment may be made by moving the lamp closer to or farther from the microscope (the position might be marked for each objective used) or by cutting paper diaphragms of fixed aperture (one for each objective used). In this instance a lamp iris would be useful.

e Lower the condenser just sufficiently to defocus the ground glass surface and render the field of illumination even.

f Observe the back aperture of the objective and open the substage condenser iris about 75 percent of the way. The final adjustment of the substage iris is made while observing the preparation; the iris should be open as far as possible, still giving good contrast.

g The intensity of illumination should be adjusted only with neutral density filters or by changing the lamp voltage.

Proper illumination is one of the most important operations in microscopy. It is easy to judge a microscopist's ability by a glance at his field of view and the objective back lens.

B Resolving Power

The resolving power of the microscope is its ability to distinguish separate details of closely spaced microscopic structures. The theoretical limit of resolving two discrete points, a distance X apart, is:

$$X \quad \frac{1.22\lambda}{2\ N.A.}$$

where λ = wavelength of light used to illuminate the specimen

N.A. = numerical aperture of the objective

Substituting a wavelength of 4,500 Angstroms and a numerical aperture of 1.3, about the best that can be done with visible light, we find that two points about 2,000A (or 0.2 micron) apart can be seen as two separate points. Further increase in resolving power can be achieved for the light microscope by using light or shorter wavelength. Ultraviolet light near 2,000 Angstroms lowers the limit to about 0.1 micron, the lower limit for the light microscope.

The numerical aperture of an objective is usually engraved on the objective and is related to the angular aperture, AA (Figure 15), by the formula:

$$N.A. = n \sin \frac{AA}{2}$$

where n = the lowest index in the space between the object and the objective.

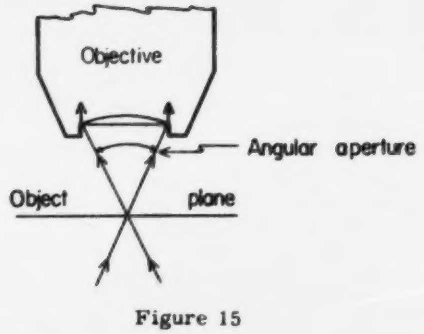


Figure 15

ANGULAR APERTURE OF MICROSCOPE OBJECTIVE

1 Maximum useful magnification

A helpful rule of thumb is that the useful magnification will not exceed 1,000 times the numerical aperture of the objective (see Tables 3 and 4). Although somewhat higher magnification may be used in specific cases, no additional detail will be resolved.

It is curious, considering the figures in the table, that most, if not all, manufacturers of microscopes furnish a 10X eyepiece as the highest power. A 10X eyepiece is useful but anyone interested in critical work should use a 15-25X eyepiece; the 5-10X eyepieces are best for scanning purposes.

2 Abbe's theory of resolution

One of the most cogent theories of resolution is due to Ernst Abbe, who suggested that microscopic objects act like diffraction gratings (Figure 16) and that the angle of diffraction, therefore, increases with the fineness of the detail. He proposed that a given microscope objective would resolve a particular detail if at least two or three transmitted rays (one direct and two diffracted rays) entered the objective. In Figure 16 the detail shown would be resolved in A and C but not in B. This theory, which can be borne out by simple experiment, is useful in showing how to improve resolution. Since shorter wavelengths will give a smaller diffraction angle, there is more chance of resolving fine detail with short wavelengths. Also, since only two of the transmitted rays are needed, oblique light and a high N. A. condenser will aid in resolving fine detail.

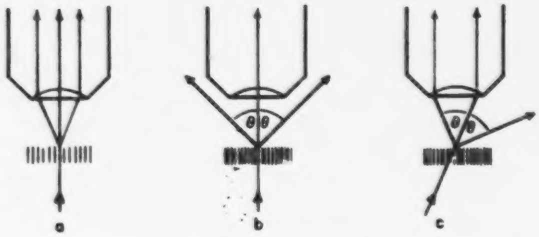


Figure 16

ABBE THEORY OF RESOLUTION

3 Improving resolving power

The following list summarizes the practical approaches to higher resolution with the light microscope:

a The specimen should be illuminated by either critical or Köhler illumination.

b The condenser should be well-corrected and have a numerical aperture as high as the objective to be used.

c An apochromatic oil-immersion objective should be used with a compensating eyepiece of at least 15X magnification. The immersion oil should have an index close to 1.515 and have proper dispersion for the objective being used.

d Immersion oil should be placed between the condenser and slide and between cover slip and objective. The preparation itself should be surrounded by a liquid having a refractive index of 1.515 or more.

e The illumination should be reasonably monochromatic and as short in wavelength as possible. An interference filter transmitting a wavelength of about 480-500 millimicrons is a suitable answer to this problem. Ideally, of course, ultraviolet light should be used to decrease the wavelength still further.

The practical effect of many of these factors is critically discussed by Loveland(2) in a paper on the optics of object space.

IV PHOTOMICROGRAPHY

A Introduction

Photomicrography, as distinct from microphotography, is the art of taking pictures through the microscope. A microphotograph is a small photograph; a photomicrograph is a photograph of a small object. Photomicrography is a valuable tool in recording the results of microscopical study. It enables the microscopist to:

1 describe a microscopic field objectively without resorting to written descriptions,

2 record a particular field for future reference,

3 make particle size counts and counting analyses easily and without tying up a microscope,

4 enhance or exaggerate the visual microscopic field to bring out or emphasize certain details not readily apparent visually,

5 record images in ultraviolet and infrared microscopy which are otherwise invisible to the unaided eye

There are two general approaches to photomicrography; one requires only a plate or film holder supported above the eyepiece of the microscope with a light-tight bellows; the other utilizes any ordinary camera with its own lens system, supported with a light-tight adaptor above the eyepiece. It is best, in the latter case, to use a reflex camera so that the image can be carefully focused on the ground glass. Photomicrography of this type can be regarded simply as replacing the eye with the camera lens system. The camera should be focused at infinity, just as the eye is for visual observation, and it should be positioned close to and over the eyepiece.

The requirements for photomicrography, however, are more rigorous than those for visual work. The eye can normally compensate for varying light intensities, curvature of field and depth of field. The photographic plate, however, lies in one plane; hence the greatest care must be used to focus sharply on the subject plane of interest and to select optics to give minimum amounts of field curvature and chromatic aberrations.

With black and white film, color filters may be used to enhance the contrast of some portions of the specimen while minimizing chromatic aberrations of the lenses. In color work, however, filters cannot usually be used for this purpose and better optics may be required.

Photomicrographic cameras which fit directly onto the microscope are available in 35-mm or up to 3-1/4 X 4-1/4 inch sizes. Others are made which accommodate larger film sizes and which have their own support independent of the microscope. The former, however, are preferred for ease of handling and lower cost. The latter system is preferred for greater flexibility and versatility and lack of vibration. The Polaroid camera has many applications in microscopy and can be used on the microscope directly but, because of its weight, only when the microscope has a vertically moving stage for focusing rather than a focusing body tube.

B Determination of Correct Exposure

Correct exposure determination can be accomplished by trial and error, by relating new conditions to previously used successful conditions and by photometry.

With the trial and error method a series of trial exposures is made, noting the type of subject, illumination, filters, objective, eyepiece, magnification, film and shutter speed. The best exposure is selected. The following parameters can be changed and the exposure time adjusted accordingly:

1 Magnification. Exposure time varies as the square of the magnification.

Example: Good exposure was obtained with a 1/10-second exposure and a magnification of 100X. If the magnification is now

200X, the correct exposure is calculated as follows:

new exposure time = old exposure time

$$\times \left(\frac{\text{new magnification}}{\text{old magnification}}\right)^2 = 1/10 \left(\frac{200}{100}\right)^2 =$$

4/10 or, say, 1/2 second.

It should be noted, however, that the above calculation can be made only when there has been no change in the illumination system including the condenser or the objective. Only changes in magnification due to changing eyepieces or bellows extension distance can be handled in the above manner.

2 Numerical aperture. Exposure time varies inversely as the square of the smallest working numerical aperture of the condenser and objective.

Example: Good exposure was obtained at 1/10 second with the 10X objective, N. A. 0. 25, at full aperture. With a 20X objective, N. A. 0. 25, at full aperture and the same final magnification, what is the correct exposure time?

new exposure time = old exposure time

$$\times \left(\frac{\text{old N. A.}}{\text{new N. A.}}\right)^2 = 1/10 \left(\frac{0.25}{0.50}\right)^2 = 1/40 \text{ or,}$$

say, 1/50 second.

It is seen that more light reaches the photographic film with higher numerical apertures at the same magnification.

3 Film. Exposure time varies inversely with the American Standards Association speed index of the film.

Example: A good picture was obtained with Eastman Tri-X film at 1/100 second. What is the correct exposure for Eastman Kodachrome II Type A. The A. S. A. speed for Tri-X is 400 and for Kodachrome II Type A Professional is 40.

new exposure time = old exposure time

$$\times \frac{\text{A. S. A. of old film}}{\text{A. S. A. of new film}} = 1/100(400/40) =$$

10/100 or 1/10 second.

4 Other parameters may be varied but the prediction of exposure time cannot be made readily. Experience and photoelectric devices are the best guides to the proper exposure.

Photoelectric devices are excellent for determining correct exposure. Since ordinary photographic exposure meters are not sensitive enough for photomicrography, more sensitive instruments, having a galvanometer or electronic amplifying circuit, are required. Some photosensitive cells are inserted in the body tube in place of the eyepiece for light intensity readings. This has the advantage of detecting the light level at a point of high intensity but does not take into account the eyepiece, the distance to the film or the film speed.

The cell may be placed just above the eyepiece so that it registers the total amount of light leaving the eyepiece. Again, the effects of film speed and the projection distance are not accounted for. The principal drawback with the total light measuring photometer is the difficulty of taking into account the area of field covered. Take, for example, a bright field in which only a few crystals appear; perhaps 1 percent of the light entering the field of view is scattered by the crystals and the photometer shows close to a maximum reading. Now assume that everything remains constant except the number of crystals and, consequently, the amount of light scattered. The photometer reading could easily drop by 50 percent, yet the proper exposure is unchanged. The situation is similar for photomicrography with crossed polars since the photometer reading depends on the intensity of illumination, on the birefringence and thickness of the crystals and on the number and size of the crystals in the field or, alternatively, on the area of the field covered by birefringent crystals. One of the best solutions to this problem is to measure the photometer reading with no preparation on the stage. A first-order red compensator or a quartz wedge is inserted when crossed polars are being used to illuminate the entire field.

An alternative is to place the cell on the ground glass where the film will be located. However, although all variables except film speed are now taken into account, measurements in the image plane have the disadvantage of requiring a more sensitive electronic photoelectric apparatus.

No matter what method is used for placing the photocell, the exposure time can be determined by the general formula:

$$\text{exposure time} = \frac{k}{\text{meter reading}}.$$

The constant k will depend on the physical arrangement and film used. To determine k for any particular system, first set up the microscope to take a picture. Record the meter reading and take a series of trial exposures. Pick out the best exposure and calculate k. Then the k which was determined holds as long as no change is made in the light path beyond the photocell, e. g. changing to a faster film or changing the projection distance. Thus the objective, condenser position or illuminator may be changed without affecting k if the cell is used as described above.

Example: With one particular arrangement of photocell and film, the meter reading is found to be 40. A series of photographs are taken at 1/2, 1/5, 1/10, 1/25 and 1/50 seconds. The photomicrograph taken at 1/5 second is judged to be the best; hence k is calculated as follows:

k = meter reading $\times$ exposure time = $40 \times 1/5 = 8$.

Assume now that a new picture is to be taken at another magnification (but with the same film and projection distance) and that the new meter reading is 16; therefore:

exposure time = k/meter reading = $8/16 = 1/2$ second.

V MICROMETRY

A Particle Size Determination

Linear distances and areas can be measured with the microscope. This permits determination of particle size and quantitative analysis of physical mixtures. The usual unit of length for microscopical measurements is the micron (1×10^{-3}mm or about 4×10^{-5}inch). Measuring particles in electron microscopy requires an even smaller unit, the millimicron (1×10^{-3} micron or 10 Angstrom units). Table 6 shows the approximate average size of a few common airborne materials.

Table 6. APPROXIMATE PARTICLE SIZE OF SEVERAL COMMON PARTICULATES

Ragweed pollen	25 microns
Fog droplets	20 microns
Power plant flyash (after precipitators)	2-5 microns
Tobacco smoke	0.2 micron (200 millimicrons)
Foundry fumes	0.1 - 1 micron (100-1000 millimicrons)

The practical lower limit of accurate particle size measurement with the light microscope is about 0.5 micron. The measurement of a particle smaller than this with the light microscope leads to errors which, under the best circumstances, increase to about ± 100 percent (usually +).

One of the principal uses of high resolving power is in the precise measurement of particle size. There are, however, a variety of approximate and useful procedures as well.

1 Methods of particle size measurement

a Knowing the magnification of the microscope (product of the magnification of objective and eyepiece), the size of particles can be estimated. For example, with a 10X eyepiece and a 16-mm (or 10X) objective, the total magnification is 100X. A particle that appears to be 10-mm at 10 inches from the eye has an actual size of 10 mm divided by 100 or 0.10 mm or 100 microns. This is in no sense an accurate method, but it does permit quick estimation of particle size; the error in this estimation is usually 10-25 percent.

b Another approximate method is also based on the use of known data. If we know approximately the diameter of the microscope field, we can estimate the percentage of the diameter occupied by the object to be measured and calculate from these figures the approximate size of the object. The size of the microscope field depends on both the objective and the ocular although the latter is a minor influence. The size of the field should be determined with a millimeter scale for each objective and ocular. If this is done, estimation of sizes by comparison with the entire field diameter can be quite accurate (5-10%).

c The movement of a graduated mechanical stage can also be used for rough measurement of diameters of large particles. Stages are usually graduated (with vernier) to read to 0.1 millimeter, or 100 microns. In practice, the leading edge of the particle is brought to one of the lines of the cross hair in the eyepiece and a reading is taken of the stage position. Then the particle is moved across the field by moving the mechanical stage in an appropriate direction until the second trailing edge just touches the cross-hair line. A second reading is taken and the difference in the two readings is the distance moved or the size of the particle. This method is especially useful when the particle is larger than the field, or when the optics give a distorted image near the edge of the field.

d The above method can be extended to projection or photography. The image of the particles can be projected on a screen with a suitable light source or they may be photographed. The final magnification, M, on the projection surface (or film plane) is given approximately by

$$M = D \times O.M. \times E.M./25$$

where O.M. = objective magnification
E.M. = eyepiece magnification
D = projection distance from the eyepiece in centimeters.

The image detail can then be measured in centimeters and the actual size computed by dividing by M. This method is usually accurate to within 2-5 percent depending on the size range of the detail measured.

e The stated magnifications and/or focal lengths of the microscope optics are nominal and vary a bit from objective to objective or eyepiece to eyepiece. To obtain accurate measurements, a stage micrometer is used to calibrate each combination of eyepiece and objective. The stage micrometer is a glass microscope slide that has, accurately engraved in the center, a scale, usually 2 millimeters long, divided into 200 parts, each part representing 0.01 millimeter. Thus when this scale is observed, projected or photographed, the exact image magnification can be determined. For example, if 5 spaces of the stage micrometer measure 6 millimeters when projected, the actual magnification is

$$\frac{6}{5\,(0.01)} = 120 \text{ times.}$$

This magnification figure can be used to improve the accuracy of method 4 above.

f The simplest procedure and the most accurate is based on the use of a micrometer eyepiece. Since the eyepiece magnifies a real image from the objective, it is possible to place a transparent scale in the same plane as the image from the objective and thus have a scale superimposed over the image. This is done by first placing an eyepiece micrometer scale disc in the eyepiece. The eyepiece micrometer has an arbitrary scale and must be calibrated with each objective used. The simplest way to do this is to place the stage micrometer on the stage and note a convenient whole number of eyepiece micrometer divisions. The value in microns for each eyepiece micrometer division is then easily computed. When the stage micrometer is removed and replaced by the specimen, the superimposed eyepiece scale can be used for accurate measurement of any feature in the specimen by direct observation, photography or projection.

2 Calibration of eyepiece micrometer

Each micrometer stage scale has divisions 100μ (0.1 mm) apart; one or two of these are usually subdivided into 10μ (0.01-mm) divisions. These form the standard against which the arbitrary divisions in the micrometer eyepiece are to be calibrated. Each objective must be calibrated separately by noting the correspondence between the stage scale and the eyepiece scale. Starting with the lowest power objective focus on the stage scale, arrange the two scales parallel and in good focus. It should be possible to determine the number of eyepiece divisions exactly equal to some whole number of divisions of the stage scale, a distance readily expressed in microns.

The calibration consists, then, of calculating the number of microns per eyepiece scale division. To make the comparison as accurate as possible, a large part of each scale must be used (see Figure 17). Let's assume that with the low power 16-mm objective 6 large divisions of the stage scale (s. m. d.) are equal to 38 divisions of the eyepiece scale. This means that 38 eyepiece micrometer divisions (e. m. d.) are equivalent to 600 microns. Hence:

$$\begin{aligned} 1 \text{ e.m.d.} &= 600/38 \\ &= 15.8\mu. \end{aligned}$$

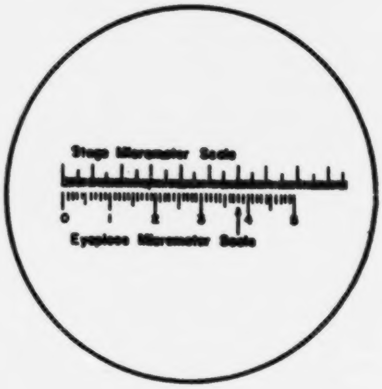


Figure 17

COMPARISON OF STAGE MICROMETER SCALE WITH EYEPIECE MICROMETER SCALE

Thus when that micrometer eyepiece is used with that 16-mm objective each division of the eyepiece scale is equivalent to 15.8μ, and it can be used to make an accurate measurement of any object on the microscope stage. A particle, for example, observed with the 16-mm objective and measuring 8.5 divisions on the eyepiece scale is 8.5 (15.8) or 135μ in diameter.

Each objective on your microscope must be calibrated in this manner.

A convenient way to record the necessary data and to calculate μ/emd is by means of a table.

Table 7

Objective	No. smd = no. emd	μ = no. emd	μ = 1 emd
32-mm	18 = 44	1800 = 44	40.9μ
16-mm	6 = 38	600 = 38	15.8μ
4-mm	1 = 30	100 = 30	3.33μ

3 Determination of particle size distribution

The measurement of particle size can vary in complexity depending on particle shape. The size of a sphere may be denoted by its diameter. The size of a cube may be expressed by the length of an edge or diagonal. Beyond these two configurations, the particle "size" must include information about the shape of the particle in question, and the expression of this shape takes a more complicated form.

Martin's diameter is the simplest means of measuring and expressing the diameters of irregular particles and is sufficiently accurate when averaged for a large number of particles. In this method, the horizontal or east-west dimension of each particle which divides the projected area into halves is taken as Martin's diameter (Figure 18).

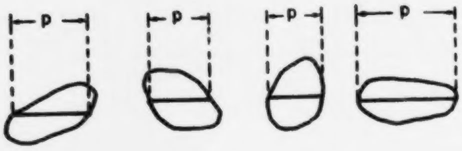


Figure 18

MARTIN'S DIAMETER

The more particles counted, the more accurate will be the average particle size. Platelike and needlelike particles should have a correction factor applied to account for the third dimension since all such particles are restricted in their orientation on the microscope slide. When particle size is reported, the general shape of the particles as well as the method used to determine the "diameter" should be noted.

Particle size distribution is determined routinely by moving a preparation of particles past an eyepiece micrometer scale in such a way that their Martin's diameter can be tallied. All particles whose centers fall within two fixed divisions on the scale are tallied. Movement of the preparation is usually accomplished by means of a mechanical stage but may be carried out by rotation of an off-center rotating stage. A sample tabulation appears in Table 8. The eyepiece and objective are chosen so that at least six, but not more than twelve, size classes are required and sufficient particles are counted to give a smooth curve. The actual number tallied (200 - 2,000) depends on particle shape regularity and the range of sizes. The size tallied for each particle is that number of eyepiece micrometer divisions most closely approximating Martin's diameter for that particle.

4 Calculation of size averages

The size data may be treated in a variety of ways; one simple, straightforward treatment is shown in Table 9. For a more complete discussion of the treatment of particle size data see Chamot and Mason's *Handbook of Chemical Microscopy*(3), page 26.

The averages with respect to number, $\bar{d}_1$; surface, $\bar{d}_3$; and weight or volume, $\bar{d}_4$, are calculated as follows for the data in Table 9.

Table 8. PARTICLE SIZE TALLY FOR A SAMPLE OF STARCH GRAINS

Size class (emd*)	Number of particles	Total
1	卌 卌 卌 1	16
2	卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 1 1 1	98
3	卌 卌	110
4	卌 1 1	107
5	卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 1	71
6	卌 卌 卌 卌 卌 卌 卌 卌 卌	45
7	卌 卌 卌 卌 1	21
8	1 1	2
		470

*emd = eyepiece micrometer divisions

$\bar{d}_1 = \Sigma nd/\Sigma n = 1758/470$

$= 3.74 \text{ emd} \times 2.82^* = 10.5\mu$

$\bar{d}_3 = \Sigma nd^3/\Sigma nd^2 = 37440/7662$

$= 4.89 \text{ emd} \times 2.82 = 13.8\mu$

$\bar{d}_4 = \Sigma nd^4/\Sigma nd^3 = 199194/37440$

$= 5.32 \text{ emd} \times 2.82 = 15.0\mu$

*2.82 microns per emd (determined by calibration of the eyepiece-objective combination used for the determination).

Cumulative percents by number, surface and weight (or volume) may be plotted from the data in Table 9. The calculated percentages, e.g.

$$\sum_{d=1}^{d} nd^4 \Big/ \sum_{d=1}^{d=15} nd^4 \times 100$$

for the cumulative weight or volume curve, are plotted against d. Finally, the specific surface, S_m, in square meters per gram, m, may be calculated if the density, D, is known; the surface average $\bar{d}_3$, is used.

If $D = 1.1$, $S_m = 6/\bar{d}_3D = 6/13.8(1.1)$

$= 0.395 m^2/g$.

Table 9. CALCULATIONS FOR PARTICLE SIZE AVERAGE

d (Aver. diam. in emd)	n	nd	nd^2	nd^3	nd^4
1	16	16	16	16	16
2	98	196	392	784	1568
3	110	330	990	2970	8910
4	107	428	1712	6848	27392
5	71	355	1775	8875	44375
6	45	270	1620	9720	58320
7	21	147	1029	7203	50421
8	2	16	128	1024	8192
	470	1758	7662	37440	199194

B Counting Analysis

Mixtures of particulates can often be quantitatively analyzed by counting the total number of particulates from each component in a representative sample. The calculations are, however, complicated by three factors: average particle size, particle shape and the density of the components. If all of the components were equivalent in particle size, shape and density then the weight percentage would be identical to the number percentage. Usually, however, it is necessary to determine correction factors to account for the differences.

When properly applied, this method can be accurate to within ± 1 percent and, in special cases, even better. It is often applied to the analysis of fiber mixtures and is then usually called a dot-count because the tally of fibers is kept as the preparation is moved past a point or dot in the eyepiece.

A variety of methods can be used to simplify recognition of the different components. These include chemical stains or dyes and enhancement of optical differences such as refractive indices, dispersion or color. Often, however, one relies on the differences in morphology, e.g. counting the percent of rayon fibers in a sample of "silk".

Example 1: A dot-count of a mixture of fiberglass and nylon shows:

nylon	262
fiberglass	168

Therefore:

$$\% \text{ nylon} = 262/(262 + 168) \times 100$$
$$= 60.9\% \text{ by number.}$$

However, although both fibers are smooth cylinders, they do have different densities and usually different diameters. To correct for diameter one must measure the average diameter of each type of fiber and calculate the volume of a unit length of each.

	aver. diam. μ	volume of 1-μ slice, μ^3
nylon	18.5	268
fiberglass	13.2	117

The percent by volume is, then:

$$\% \text{ nylon} = \frac{262 \times 268}{(262 \times 268)+(168 \times 117)} \times 100$$
$$= 78.1\% \text{ by volume.}$$

Still we must take into account the density of each in order to calculate the weight percent.

If the densities are 1.6 for nylon and 2.2 for glass then the percent by weight is:

$$\%\ \text{nylon} = \frac{262 \times 268 \times 1.6}{(262 \times 268 \times 1.6)+(168 \times 117 \times 2.2)} \times 100$$

$$= 72\%\ \text{by weight.}$$

Example 2: A count of quartz and gypsum shows:

quartz	283
gypsum	467

To calculate the percent by weight we must take into account the average particle size, the shape and the density of each.

The average particle size with respect to weight, $\bar{d}_4$, must be measured for each and the shape factor must be determined. Since gypsum is more platelike than quartz each particle of gypsum is thinner. The shape factor can be approximated or can be roughly calculated by measuring the actual thickness of a number of particles. We might find, for example, that gypsum particles average 80% of the volume of the average quartz particle; this is our shape factor. The final equation for the weight percent is:

$$\%\ \text{quartz} = \frac{283 \times \pi\bar{d}_4/6 \times Dq}{238 \times \pi\bar{d}_4/6 \times Dq + 467 \times \pi\,\bar{d}_4^*/6 \times 0.80 \times Dg} \times 100$$

where Dq and Dg are the densities of quartz and gypsum respectively; 0.80 is the shape factor and $\bar{d}_4$ and $\bar{d}_4^*$ are the average particle sizes with respect to weight for quartz and gypsum respectively.

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REFERENCES

1 Bunn, C.W. Crystal Growth from Solution. Discussions of the Faraday Society No. 5. 132. Gunery and Jackson. London. (1949).

2 Loveland, R.P., J. Roy. Micros. Soc. 79, 59. (1960).

3 Chamot, Emile Monnin, and Mason, Clyde Walter. Handbook of Chemical Microscopy, Vol. 1, third ed. John Wiley and Sons, New York (1959).

STRUCTURE AND FUNCTION OF CELLS

I INTRODUCTION

What are cells? Cells may be defined as the basic structural units of life. The cell has many different parts which carry on the various functions of cell life. These are called organelles ("little organs").

A The branch of biology which deals with the form and structure of plants and animals is called "Morphology." The study of the arrangement of their several parts is called "anatomy", and the study of cells is called "cytology".

B There is no "typical" cell, for cells differ from each other in detail, and these differences are in part responsible for the variety of life that exists on the earth.

II FUNDAMENTALS OF CELL STRUCTURE

A How do we recognize a structure as a cell? We must look for certain characteristics and/or structures which have been found to occur in cells. The cell is composed of a variety of substances and structures, some of which result from cellular activities. These include both living and non-living materials.

1 Non-living components include:

a A "cell wall" composed of cellulose may be found as the outermost covering of many plant cells.

b "Vacuoles" are chambers in the protoplasm which contain fluids of different densities (i.e., different from the surrounding protoplasm).

2 The "living" parts of the cell are called "protoplasm." The following structures are included:

a A thin "cell membrane" is located just inside the cell wall. This membrane may be thought of as the outermost layer of protoplasm.

b In plant cells the most conspicuous protoplasmic structures are the "chloroplasts", which contain highly organized membrane systems bearing the photosynthetic pigments (chlorophylls, carotenoids, and xanthophylls) and enzymes.

c The "nucleus" is a spherical body which regulates cell function by controlling enzyme synthesis.

d "Granules" are structures of small size and may be "living" or non-living" material.

e "Flagella" are whip-like structures found in both plant and animal cells. The flagella are used for locomotion, or to circulate the surrounding medium.

f "Cilia" resemble short flagella, found almost exclusively on animal cells. In the lower animals, cilia are used for locomotion and food gathering.

g The "pseudopod", or false foot, is an extension of the protoplasm of certain protozoa, in which the colloidal state of the protoplasm alternates from a "sol" to a "jel" condition from time to time to facilitate cell movement.

h "Ribosomes" are protoplasmic bodies which are the site of protein synthesis. They are too small (150 Å in diameter) to be seen with a light microscope.

i "Mitochondria" are small membranous structures containing enzymes that oxidize food to produce energy transfer compounds (ATP).

B How basic structure is expressed in some major types of organisms.

We can better visualize the variety of cell structure by considering several specific cells.

1 Bacteria have few organelles, and are so minute that under the light microscope only general morphological types (i.e., the three basic shapes; rods, spheres, and spirals) can be recognized. The following structures have been defined:

a The "capsule" is a thick protective covering of the cell exterior, consisting of polysaccharide or polypeptide.

b The cell wall and plasma membrane are present.

c Although no well defined nucleus is visible in bacterial cells, the electron microscope has revealed areas of deoxyribose nucleic acid (DNA) concentration. This substance is present within the nucleus of of higher cells, and is the genetic or hereditary material.

d Some types of bacteria contain a special type of chlorophyll (bacteriochlorophyll) and carry on photosynthesis.

2 The blue-green algae are similar to the bacteria in structure, but contain the photosynthetic pigment chlorophyll a.

a Like the bacteria, these forms also lack an organized nucleus (the nuclear region is not bounded by a membrane).

b The chlorophyll-bearing membranes are not localized in distinct bodies (chloroplasts), but are dispersed throughout the cell.

c Gas-filled structures called "pseudovacuoles" are found in some types of blue-greens.

3 The green algae as a group include a great variety of structural types, ranging from single-celled non-motile forms to large motile colonies. Some types are large enough to resemble higher aquatic plants.

a The chloroplasts are modified into a variety of shapes and are located in different positions. Examples of chloroplast shape and position are:

1) Parietal - located on the periphery of the cell; usually cup-shaped and may extend completely around the inner surface of the plasma membrane.

2) Discoid - also located on the periphery of the cell, but are plate-shaped; usually many per cell.

3) Axial - lying in the central axis of the cell; may be ribbon-like or star-shaped.

4) Radial - have arms or processes that extend outward from the center of the cell (radiate), reaching the plasma membrane.

5) Reticulate - a mesh-like network that extends throughout volume of the cell.

b Located in the chloroplasts may be dense, proteinaceous, starch-forming bodies called "pyrenoids".

4 The flagellated algae possess one-to-eight flagella per cell. The chloroplasts may contain brown and/or red pigments in addition to chlorophyll.

a Reserve food may be stored as starch (Chlamydomonas) paramylon (Euglena), or as oil.

5 The protozoa are single-celled animals which exhibit a variety of cell structure.

a The amoebae move by means of pseudopodia, as described previously.

b The flagellated protozoa (Mastigophora) possess one or more flagella.

c The ciliates are the most highly modified protozoans. The cilia may be more or less evenly distributed over the entire surface of the cell, or may be localized.

III FUNCTIONS OF CELLS

What are the functions of cells and their structural components? Cellular function is called "life", and life is difficult to define. Life is characterized by processes commonly referred to as reproduction, growth, photosynthesis, etc.

A Microorganisms living in surface waters are subjected to constant fluctuations in the physical and chemical characteristic of the environment, and must constantly modify their activities.

1 The cell requires a cource of chemical energy to carry on life processes and successfully compete with other organisms. Plant cells may obtain this energy from light, which is absorbed by chlorophyll and converted into ATP or food reserves, or from the oxidation of food stuffs. Animal cells obtain energy only from the oxidation of food.

2 Cells must obtain raw materials from the environment in order to grow and carry out other life functions. Inorganic and organic materials may be taken up by passive diffusion or by "active transport". In the later process, energy is used to build up and maintain a higher concentration of a substance (such as phosphate) inside the cell than is found outside. Algae are able to synthesis organic matter from inorganic raw materials (carbon dioxide and water), with the aid of energy derived from light, whereas animal cells must obtain their organic matter "ready-made" by consuming other organisms, organic debris, or dissolved organics.

IV SUMMARY

The cell is made up of many highly specialized substructures. The types of substructures present, and their appearance (shape, color, etc.) are very important in understanding the role of the organism in the aquatic community, and in classification.

REFERENCES

1 Bold, H. C. Cytology of algae. In: G. M. Smith, (ed.), Manual of Phycology. Ronald Press. 1951.

2 Bourne, Geoffry H., ed. Cytology and Cell Physiology. 3rd ed. Academic Press. 1964.

3 Brachet, Jean. The Living Cell. Scientific American. 205(3). 1961.

4 Corliss, John O. Ciliated Protozoa. Pegamon. 1961.

5 Fritsch, F. E. The structure and reproduction of the algae. Cambridge Univ. Press. 1965.

6 Frobisher, M. Fundamentals of microbiology. 7th edition. W. B. Saunders Co., Philadelphia. 1962.

7 Round, F. E. The biology of the algae. St. Martin's Press. New York. 1965.

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TYPES OF ALGAE

I INTRODUCTION

A Algae in general may be defined as small pigmented plant-like organisms of relatively simple structure. Actually the size range is extreme: from only a few microns to over three hundred feet in length. Commonly observed examples include the greenish pond scum or frog spittle of freshwater ponds, much of the golden brown slime covering rocks in a trout stream, and the great marine kelps and seaweeds. Large freshwater forms as Nitella and Chara or stonewort are also included.

B Algae approach ubiquity in distribution. In addition to the commonly observed bodies of water, certain algae also live in such unlikely places as thermal springs, the surface of melting snow, on the hair of the three toed sloth in Central America, and in conjunction with certain fungi to form lichens.

III ALGAE WILL BE GROUPED FOR THE SAKE OF CONVENIENCE INTO FOUR GENERAL TYPES:

A Blue-greens (See plate: Blue-Green Algae, Cyanophyceae). This is a valid technical group. The size range is not very great, some being so small as to approach the size range of the bacteria.

1 These are the only algae in which the pigments are not localized in definite bodies but dissolved throughout the cell. Blue, red, or other pigments are present in addition to chlorophyll thus giving the cells a bluish green, yellow, or red color, at least enmasse.

2 The nucleus lacks a nuclear membrane.

3 Tend to achieve nuisance concentrations more frequently in the warm summer months and in the richer waters.

4 Vegetative reproduction, in addition to cell division, includes the formation of "hormogones," or short specifically delimited sections of trichomes (filaments).

5 Spores of three types are encountered:

a Akinetes are usually larger, thick walled resting spores.

b Heterocysts appear like empty cell walls, but are actually filled with protoplasm, have occasionally been observed to germinate.

c Endospores, also called "gonidia" or conidia, are formed by repeated division of the protoplast within a given cell wall. Present in only a few genera.

6 Some common examples of blue-green algae are:

Anacystis (Microcystis or Polycystis), Anabaena, Aphanizomenon, and Oscillatoria

B The Pigmented flagellates (in contrast to the non-pigmented or animal-like flagellates) are a heterogeneous collection of motile forms from several different algal groups (See plate: Flagellated algae).

1 There may be one, two, four, or more flagella per cell.

2 There is a well organized nucleus.

3 A light-sensitive red eyespot usually present.

4 The chlorophyll is contained in one or more distinctive bodies called plastids.

5 Two or more cells may be associated in a colony.

6 Non-motile life history stages may be encountered.

7 Masses of stored starch called pyrenoid bodies are often conspicuous.

8 Some examples of pigmented flagellates are: Euglena, Phacus, Chlamydomonas, Gonium, Volvox, Peridinium, Ceratium Mallomonas, Synura and Dinobryon.

C The Non-motile green algae constitute another heterogeneous assembly of unrelated forms (See plate: Non-Motile Green Algae)

1 Like the flagellates they have well organized nuclei and chloroplasts. The shape of the chloroplast is often distinctive.

2 They lack flagella or any other locomotor device.

3 There is extreme structural variation among the group.

4 Some types tend to occur as a general planktonic mass or bloom," often in combinations of two or more species.

Some examples are: Sphaerocystis, Pediastrum, Scenedesmus, and the desmid Cosmarium.

5 Threadlike (filamentous) green algae may form masses or blankets, cutting off light, and reducing water circulation. They also add considerably to the total mass of organic matter. Some examples of this type are: Spirogyra, Hydrodictyon, Cladophora, Oedogonium, and Chara.

D The Diatoms constitute another valid technical group (See plate: Diatoms-Bacillariophyceae).

1 In appearance, they are geometrically regular in shape. The presence of a brownish pigment in addition to the chlorophyll gives them a golden to greenish color.

2 Motile forms have a distinctive hesitating progression.

3 The most distinctive structural feature is the two-part shell (frustule) composed of silicon dioxide (glass).

a One part fits inside the other as the two halves of a pill box, or a petri dish.

b The surface of these shells are sculptured with minute pits and lines arranged with geometrical perfection.

c The view from the side is called the "girdle view," that from above or below, the "valve view."

4 There are two general shapes of diatoms, circular (centric) and elongate (pennate). The elongate forms may be motile, the circular ones are not.

5 Diatoms may associate in colonies in various ways.

6 Examples of diatoms frequently encountered are: Stephanodiscus Cyclotella, Asterionella, Fragilaria, Tabellaria, Synedra, and Nitzschia.

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KEY FOR IDENTIFICATION OF GROUPS OF FRESHWATER ALGAE

Beginning with "1a" and "1b", choose one of the two contrasting statements and follow this procedure with the "a" and "b" statements of the number given at the end of the chosen statement. Continue until the name of the algal group is given instead of another key number.

1a. Plastid (separate color body) absent, complete protoplast pigmented; generally blue-green; iodine starch test* negative ------------------------------- Blue-green algae

1b. Plastid or plastids present; parts of protoplast free of some or all pigments; generally green, brown, red, etc., but not blue-green; iodine starch test* positive or negative-------- 2

2a. Cell wall permanently rigid (never showing evidence of collapse), and with regular pattern of fine markings (striations, etc.); plastids brown to green; iodine starch test* negative; flagella absent; wall of two essentially similar halves, one placed over the other as a cover-----------------Diatoms

2b. Cell wall, if present, capable of sagging, wrinkling, bulging or rigidity, depending on existing turgor pressure of cell protoplast; regular pattern of fine markings on wall generally absent; plastids green, red, brown, etc.; iodine starch test* positive or negative; flagella present or absent; cell wall continuous and generally not of two parts----------------- 3

3a. Cell or colony motile; flagella present (often not readily visible); anterior and posterior ends of cell different from one another in contents and often in shape-------------------Flagellate algae

3b. Non-motile; true flagella absent; ends of cells often not differentiated-------------------Green algae and associated forms

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*Add one drop Lugol's (iodine) solution, diluted 1-1 with distilled water. In about 1 minute, if positive, starch is stained blue and, later black. Other structures (such as nucleus, plastids, cell wall) may also stain, but turn brown to yellow.

CMP

COMPARISON OF FOUR MAJOR GROUPS OF ALGAE

	Blue-Green	Pigmented flagellates	Greens	Diatoms
Color	Blue-Green (Brown)	Green Brown	Green	Brown (Light-Green)
Location of pigment	Throughout cell	In plastids	In plastids	In plastids
Starch	Absent	Present or Absent	Present	Absent
Slimy coating	Present	Absent in most	Absent in most	Absent in most
Nucleus	Absent	Present	Present	Present
Flagellum	Absent	Present	Absent	Absent
Cell Wall	Inseparable from slimy coating	Thin or Absent	Semi-rigid smooth or with spines	Very rigid, with regular markings
"Eye" spot	Absent	Present	Absent	Absent

BLUE-GREEN ALGAE

I WHAT ARE THE BLUE-GREEN ALGAE?

The blue-green algae (Myxophyceae) comprise that large group of microscopic organisms living in aquatic or moist habitats, carrying on photosynthesis and having differentiation of cells which is a little more complex than bacteria, and simpler than all of the other plants called algae.

II WHY ARE THEY CALLED BLUE-GREEN:

In addition to the green photosynthetic pigment (chlorophyll-a) they always have a blue pigment (phyocyanin-c) which tends to give the cushions or mats they may form a blue-green tinge.

III WHERE ARE THE BLUE-GREENS FOUND?

Some are free floating (pelagic and planktonic), others grow from submerged or moist soil, rocks, wood and other objects in both freshwater and marine habitats.

IV WHAT ARE SOME OF THEIR GENERAL CHARACTERISTICS?

Some are gelatinous masses of various shapes floating in water. Others, microscopic in size, grow in great numbers so as to color the water in which they live. Structurally their cells are similar to bacteria. Their protoplasts may be sheathed or imbedded in gelatin, making them slimy. Cells of blue-green algae are without organized nuclei, central vacuoles, or cilia and flagella.
No sexual reproduction is known. Asexual reproduction may be effected by fragmentation, in which case special separation devices are formed (dead cells, and heterocysts). Some species are preserved over unfavorable periods by special spores (akinetes and endospores).

V OF WHAT IMPORTANCE ARE BLUE-GREEN ALGAE?

They have both positive and negative economic significance. Because they can convert radient energy into chemical energy, they are producers forming a first link at the base of the food chain. Because many very intricate nutritional relationships exist among the myraids of organisms it is difficult to know the value of the blue-greens. However, people who know what the blue-greens can do to drinking and recreational water classify them as of negative economic importance, because they are often nuisances when they impart color, bad odors, and fishy tastes, or toxins. Some of them can foul pipes and clog filters.

VI WHEN ARE THEY MOST COMMON?

They are widely distributed in time and space, but tend to reach nuisance concentrations more frequently in the late summer and in eutrophic waters.

VII WHAT DO BLUE-GREEN ALGAE DO FOR A LIVING?

The pioneer-forms are of great ecological importance because they live in habitats frequented by few other forms of live, synthesizing organic substances and building substrata that can support other kinds of life.

A Some blue-greens live in association with other organisms as symbionts. Still others are found in polluted waters, because they are able to exist in habitats poor in oxygen. The growth of these kinds of algae under such conditions tends to make a polluted condition worse.

B On the other hand some species should be promoted because they provide oxygen and food through photosynthesis. The first evident product of photosynthesis is glycogen, and is the cause of the brown coloration with the iodine test. Some of the glycogen is used to produce glycoproteins. The gelatinous sheath is composed of pectic substances, cellulose and related compounds.

C When blue-greens mat at the surface of the water the increased lighting may be too strong, resulting in a kill. At this time they may turn from a blue-green to a yellow-green color. Here they decompose in mass. The resulting intermediate products of decomposition may be highly undesirable, because of bad looks, four odors, bad tastes and toxins. Under these conditions the BOD may produce conditions not unlike raw sewage.

VIII WHAT DO BLUE-GREEN ALGAE LOOK LIKE UNDER THE MICROSCOPE?

A A cross section of a typical cell would show an outside nonliving gelatinous layer surrounding a woody cell wall, which is bulging from turgor pressure from the cell (plasma) membrane, pushing the wall outwardly. The protoplasm, contained within the plasma membrane, is divided into two regions. The peripheral pigmented portion called chromatoplasm, and an inner centroplasm, the centroplasm contains chromatins, which is also known as in incipient nucleus or central body, containing chromosomes and genes. Structures (chromatophores or plastids) containing pigments have not been found in the blue-greens. The photosynthetic pigments are dissolved in the peripheral cytoplasm, which is known as the chromatoplasm.

B A simple way to understand the cross section would be to compare it with a doughnut, with the hole representing the colorless central body or incipient nucleus, which houses the chromatoplasm, having the characteristic blue-green color from its dissolved photosynthetic pigments.

IX WHAT CAUSES THESE FOUL-TO-SMELL UNSIGHTLY BLOOMS?

When the protoplasts become sick or old they may develop a great number of "pseudovacuoles" filled with gas. These gas bubbles make the algae buoyant in such a way that they may "flower" or bloom by rising to the surface (planktonic, healthy blue-greens normally possess pseudovacuoles, which are here excepted). Soon they begin to stink because of the odors produced from putrefaction. The lack of dissolved oxygen during this period may affect other organisms.

X ARE ALL BLOOMS PUTREFACTIVE?

No. Healthy blooms are produced by myraids of cells living near the surface of the water at times when environmental conditions are especially favorable for them. Putrefactive blooms are usually from masses of algae undergoing degradation.

XI WHAT ARE SOME OF THE MAJOR KINDS OF BLUE-GREENS?

Most species of blue-greens may be placed into two major groups: the nonfilamentous (coccoid) forms, and the filamentous forms. See the set of drawings following this treatment to get a graphic concept of the two groups.

XII WHAT ARE SOME OF THE MORE DISTINCTIVE FEATURES OF BLUE-GREENS?

A In comparing the blue-greens with other algae it is easier to tell what they do not possess than what they do. They do not have chromatophores or plastids, cilia flagella, organized nuclei, gametes, central vacuoles, chlorophyll-b, or true starch.

B Many of the filamentous forms, especially the Oscillatoriaceae, exhibit an unexplained movement. When the filamentous forms are surrounded by a gelatinous sheath the row of cells inside is called a trichome, and the trichome with its enclosing sheath is called a filament. There may be more than one trichome within a sheath.

True branching occurs when a cell of the series divides lengthwise and the outer-formed cell adds cells to form a true branch. However, two or more trichomes within a single sheath may be so arranged that though they appear to be branches, their cells actually have all divided in the same plane, and the trichomes have pushed out from growth to form false branching, as in Tolypothrix.

C An occasional reticulated or bubbly appearance is referred to as pseudovacuolation, and en mass imparts a pale, yellowish color to the algae. Under low powers these vacuoles appear dark, under higher magnifications they are reddish.

D Vegetative reproduction in addition to cell division for the unicellular forms, is by special kinds of fragmentation. This includes the formation of hormogones, which are specifically delimited sections of trichomes, and are characteristic of some taxonomic entities.

E Spores of three types are encountered.

1 Akinetes are usually larger, nonmotile, thick-walled resting spores.

2 Heterocysts appear like empty cell walls, but are filled with colorless protoplasm and have been occasionally observed to germinate.

3 Endospores, also called gonidia, are formed by a repeated division of the protoplast within a cell wall container.

XIII WHAT ARE SOME EXAMPLES OF BLUE-GREEN ALGAE?

A Anacystis (Microcystis) is common in hard waters.

1 Colonies are always free floating.

2 Their shapes may be roughly spherical or irregular, microscopic or macroscopic.

3 The gelatinous matrix may be extremely transparent, easily broken up on preservation.

4 They frequently contain pseudovacuoles.

B Anabaena is an example of a filamentous form.

1 Filaments may occur singly or in irregular colonies, and free floating or in a delicate nucous matrix.

2 Trichomes have practically the same diameter throughout; may be straight, spiral, or irregularly contorted.

3 Cells are usually spherical, or barrel shaped, rarely cylindrical and never discoid.

4 Heterocysts are usually the same shape but are slightly larger than the vegetative cells.

5 Akinetes are always larger than the vegetative cells, roughly cylindrical, and with rounded ends.

6 It may be readily distinguished from Nostoc by the lack of a firm gelatinous envelope.

7 It may produce an undesirable grassy, moldy or other odor.

C Aphanizomenon is a strictly planktonic filamentous form.

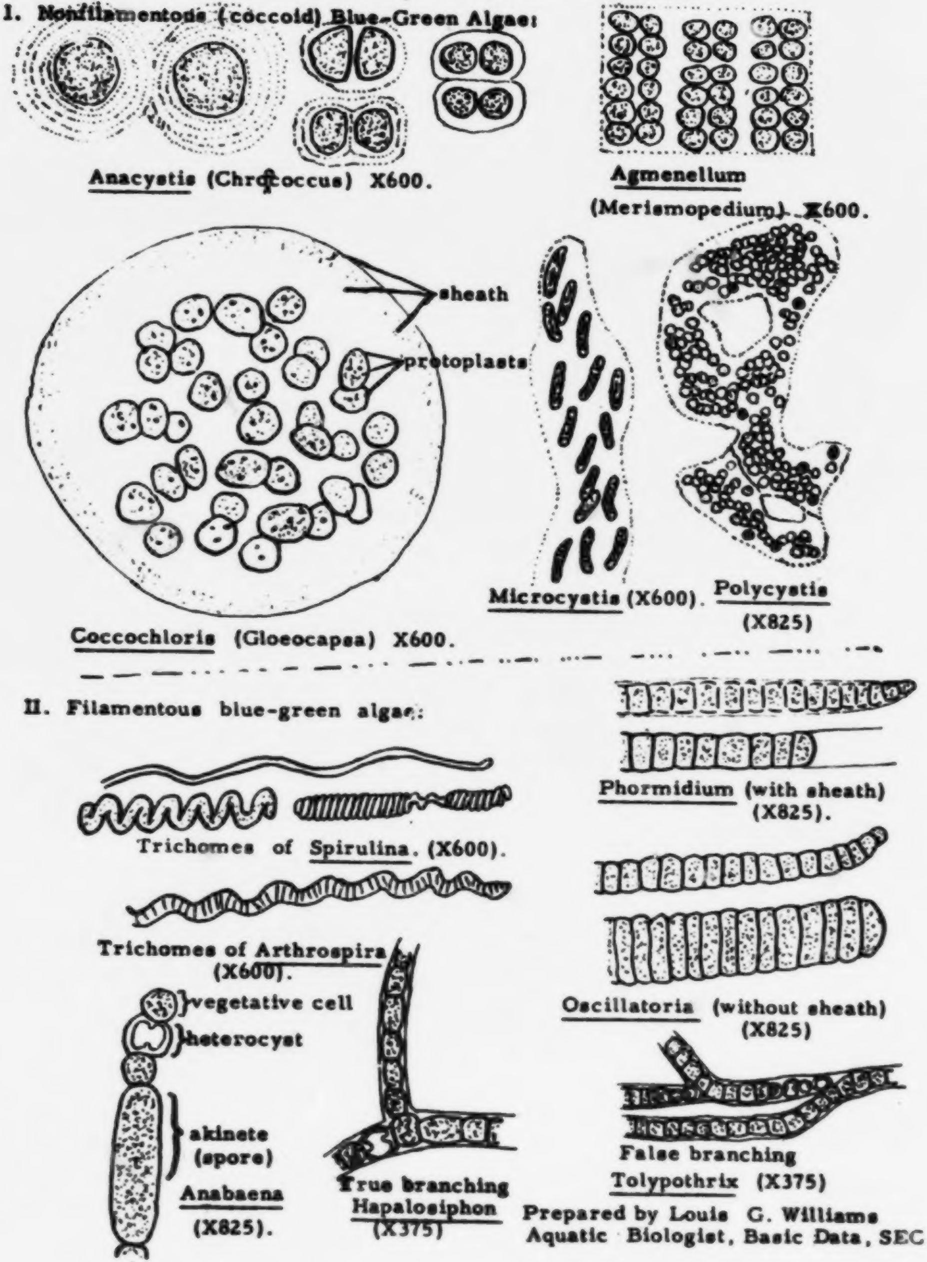
1 Trichomes are relatively straight, and laterally joined into loose macroscopic free-floating flake-like colonies.

2 Cells are cylindrical or barrel shaped, longer than broad.

3 Heterocysts occur within the filament (i.e., not terminal).

4 Akinetes are cylindrical and relatively long.

SOME BLUE-GREEN ALGAE


I. Nonfilamentous (coccoid) Blue-Green Algae:
Anacystis (Chroococcus) X600.
Agmenellum
(Merismopedium). X600.
sheath
protoplasts
Coccochloris (Gloeocapsa) X600.
Microcystis (X600).
Polycystis
(X825)
II. Filamentous blue-green algae:
Trichomes of Spirulina. (X600).
Trichomes of Arthrospira
(X600).
Phormidium (with sheath)
(X825).
Oscillatoria (without sheath)
(X825)
vegetative cell
heterocyst
akinete
(spore)
Anabaena
(X825).
True branching
Hapalosiphon
(X375)
False branching
Tolypothrix (X375)

Prepared by Louis G. Williams
Aquatic Biologist, Basic Data, SEC.

5 Often imparts grassy or nasturtium-like odors to water.

D Oscillatoria is a large and ubiquitous genus.

1 Filaments may occur singly or interwoven to form mats of indefinite extent.

2 Trichomes are unbranched, cylindrical, and practically without sheaths.

3 Species with narrow trichomes have long cylindrical cells while those with broader trichomes have short broad cells.

4 No heterocysts or akinetes are known in Oscillatoria. It reproduces by fragmentation from hormongonia only.

5 Live species exhibit "oscillatoria" movements, which are oscillating.

6 Species of Oscillatoria may be readily distinguished from Lyngbya by the absence of a sheath.

E Nodularia is an occasional producer of blooms.

1 Vegetative cells, heterocysts, and even the akinetes are broader than long.

2 Trichomes are practically the same diameter throughout.

3 Sheaths are usually distinct, fairly firm, and with a single trichome.

REFERENCES

1 Bartsch, A. F. (ed.) Environmental Requirements of Blue-Green Algae. FWPCA. Pacific Northwest Water Laboratory, Corvallis, Oregon. 111 pp. 1967.

2 Desikachary, T. V. Cyanophyta, Indian Council Agric. Res. New Delhi. 1959.

3 Drouet, Francis. Mxyophyceae. Chapter 5 in Edmondson. Freshwater Biology. p. 95-114. Wiley. 1959.

4 Drouet, Francis. Revision of the Classification of the Oscillariaceae. Monograph 15. Acad. Nat. Sci. Phil. 370 pp. 1968.

5 Jackson, Daniel F. (ed.) Algae, Man, and the Environment. Univ. Syracuse Press. 554 pp. 1968.

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GREEN AND OTHER PIGMENTED FLAGELLATES

I INTRODUCTION

A A flagellate is a free swimming cell (or colony) with one or more flagella.

B Motile flagellated cells occur in most (not all) great groups of plants and animals.

C Out main concern will be with "mature" flagellated algae.

II THE STRUCTURE OF A PIGMENTED OR PLANT-LIKE FLAGELLATE

A There is a well organized nucleus.

B The flagellum is a long whip-like process which acts as a propeller.

1 It has a distinctive structure.

2 There may be one or several per cell.

C The chlorophyll is contained in one or more chloroplasts.

D Two or more cells may be associated in a colony.

E Non-Motile Life history stages may be encountered.

F Size is of little use in identification.

G Pyrenoid bodies are often conspicuous.

III The Euglenophyta or *Euglena*-like algae (Figures 1-4) are almost exclusively single celled free swimming flagellates. Nutrition may be holophytic, holozoic, or saprophytic, even within the same species. Referred to by zoologists as mastigophora; many animal like forms are parasitic or commensalistic. Food reserves of plant-like forms are as paramylin (an insoluble carbohydrate) and fats (do not respond to starch test). Thick walled resting stages (cysts) are common.

"Metabolic movement" characteristics of some genera (*Euglena*).

Eyespot usually present in anterior end, rarely more than one flagellum.

A *Euglena* is a large genus with pronounced metabolic movement (Figure 1).

1 Cells spindle shaped

2 Single flagellum

3 Eyespot usually present

4 Chloroplasts numerous, discoid to band shaped

5 *E. sanguinea* has red pigment.

6 *E. viridis* generally favors water rich in organic matter.

7 *E. gracilis* is less tolerant of pollution.

B *Phacus* cells maintain a rigid shape (Figure 2).

1 Often flattened and twisted, with pointed tip or tail end.

2 Cell wall (periplast) often marked with fine ridges.

3 *P. pyrum* favored by polluted water.

4 *P. pleuronectes* relatively intolerant of pollution.

C *Trachelomonas* cells surrounded by a distinct shell (lorica) with flagellum sticking through hole or collar (Figure 4).

1 Surface may be smooth or rough

2 Usually brown in color

3 Some species such as *T. cerebea* known to clog filters

D Lepocinclis has rigid naked cells with longitudinal or spiral ridges (Figure 3).

  1 Cells uncompressed, elipsoidal to oval (in contrast to phacus)

  2 Only two species with pointed tails

  3 L. texta often associated with waters of high organic content

IV The Chlorophyta or grass green algae (Figures 5-9) are the largest and most varied group. Non-flagellated forms predominate but many conspicuous flagellates are included. Food reserves are usually stored as starch which is readily identified with iodine. Usually two flagella of equal length are present. More planktonic forms are included than in any other group, predominating in the late spring and early autumn.

The cell is typically surrounded by a definite wall and usually has a definite shape. Cell pigments closely resemble those of higher plants, but some have accessory pigments and a few forms have little or none. The chloroplasts always have a shape characteristic of the genus.

The flagellated chlorophyta are contained in the Order Volvocales, the Volcocine algae. All are actively motile during vegetative phases. May be unicellular or colonial. All have an eyespot near the base of the flagella. Colonies may range from a simple plate (Gonium sociale) to a complete hollow sphere (Volvox spp).

A Chlamydomonas is a solitary free swimming genus (Figure 5).

  1 Species range from cylindrical to pearshaped.

  2 Some species have a gelatinous sheath.

  3 There are two flagella inserted close together.

  4 Generally favored by polluted waters.

B Carteria resembles Chlamydomonas very closely except that it has four flagella instead of two. Generally favored by polluted water (Figure 7).

C Phacotus usually has free swimming biflagellate cells surrounded by biconcave envelopes resembling two clam shells. These are usually sculptured, dark colored, and impregnated with calcium carbonate.

  1 The eyespot ranges from anterior to posterior.

  2 Several daughter cells may be retained within the old envelopes of the parent cell.

  3 A clean water indicator.

D Chlorogonium is a distinctive genus in which the cell is fusiform, the tail end pointed, and the anterior end slightly blunt (Figure 6).

  1 The two flagella only about half as long as the cell.

  2 The cell wall is rather delicate.

  3 An eyespot usually present near the anterior end.

  4 Favored by pollution.

E Gonium colonies typically have 4 to 32 cells arranged in a plate (Figure 8).

  1 The cells are imbedded in a gelatinous matrix.

  2 Sixteen celled colonies move through the water with a somersault-like motion.

  3 Four and eight celled colonies swim flagella end first.

  4 Gonium pectorale is typically a plankton form.

F Pandorina colonies range up to 32 cells, usually roughly spherical (Figure 9).

1 Cells arranged in a hollow sphere within a gelatinous matrix.

2 Often encountered especially in hard-water lakes, but seldom abundant.

3 P. morum may cause a faintly fishy odor.

G Eudorina has up to 64 cells in roughly spherical colonies.

1 The cells may be deeply imbedded in a gelatinous matrix.

2 Common in the plankton of soft water lakes.

3 E. elegans is widely distributed.

4 May cause faintly fishy odor.

H Pleodorina has up to 128 cells located near the surface of the gelatinous matrix. It is widespread in the United States.

I Volvox rarely has less than 500 cells per colony.

1 Central portion of the mature colony may contain only water.

2 Daughter colonies form inside the parent colony.

3 V. aureus imparts a fishy odor to the water when present in abundance.

J Chlamydobotrys has "mulberry shaped" colonies, with biflagellate cells alternately arranged in tiers of four each. (Spondylomorum has quadriflagellate cells).

1 There is no enveloping sheath.

2 C. stellata is favored by pollution.

V The Pyrrhophyta includes principally the armored or dinoflagellates (Dinophyceae) (Figures 14-16). This group is almost exclusively flagellated and is characterized by chromatophores which are yellow-brown in color. Food reserves are stored as starch or oil. Naked, holozoic, and saprozoic representatives are found. Both "unarmored", and "armored" forms with chromatophores are found to ingest solid food readily, and holozoic nutrition may be as important as holophytic.

The great majority have walls of cellulose consisting of a definite number of articulated plates which may be very elaborate in structure. There is always a groove girdling the cell in which one flagellum operates, the other extends backward from the point of origin.

Most of the dino-flagellates are marine and some are parasitic. There are six fresh water genera of importance in this country.

A Gymnodinum species are generally naked except for a few freshwater species.

G. brevis (marine) is a toxic form considered to be responsible for the "red tide" episodes in Florida and elsewhere.

B Species of Gonyaulax (catanella and tamarensis) are responsible for the paralytic shellfish poisoning.

C Ceratium is distinctive in that the anterior and posterior ends are continued as long horns (Figure 16).

1 Seasonal temperature changes have a pronounced effect on the shape of the cells of this species.

2 C. hirudinella in high concentration is reported to produce a "vile stench".

D Peridinium is a circular, oval, or angular form, depending on the view (Figure 15).

1 Cell wall is thick and heavy.

2 Plates are usually much ornamented.

3 P. cinctum has been charged with a fishy odor.

VI The Division Chrysophyta contains two classes which include flagellates, the Xanthophyceae or Heterokontae (yellow-green algae) and the Chrysophyceae (golden-green algae) (Figures 10-13). The third class, the diatoms (Bacillarieae or Bacillariophyceae), is not flagellated.

A None of the Xanthophyceae are included in the present discussion.

B The Chrysophyceae possess chromatophores of a golden brown color, usually without pyrenoids. Food reserves are stored as fats and leucosin. One or two flagella; if two, they may be of equal or unequal length. Internal silicious cysts may be formed. Tend to occur in relatively pure water. Both holozoic and holophytic types of nutrition are found. Certain minute forms considered to be highly sensitive to pollution.

1 Mallomonas is a solitary, free swimming genus with one flagellum (Figure 13).

a Covered with silicious plates, many of which bear long silicious spines.

b Tends to inhabit clear water lakes at moderate depths.

c M. caudata imparts a fishy odor to the water.

2 Chrysococcus cells are minute, with two yellowish brown chromatophores and one flagellum.

a Droplets of stored oil present

b Lorica distinct

c C. rufescens a clean water form

3 Chromulina has a single flagellum, may accumulate single large granule of leucosin at posterior end of cell (Figure 10).

C. rosanoffii is a clean water indicator.

4 Synura is a biflagellate form growing in radially arranged, naked colonies (Figure 11).

a Flagella equal in length

b Cells pyriform or egg shaped

c S. uvella produces a cucumber or muskmelon odor

5 Uroglenopsis forms free swimming colonies of approximately spherical biflagellate cells embedded near the periphery of a roughly spherical gelatinous matrix.

a Flagella are unequal in length.

b U. americana may range up to .5 mm in diameter, and contain 1000 or more cells.

c U. am. also causes strong fishy odor.

6 Dinobryon may be solitary or colonial, free floating or attached. Colonies are arborescent (Figure 12).

a Cells attached to bottom of open roughly cylindrical lorica or sheath.

b Two flagella of unequal length.

c Conspicuous eyespot usually present.

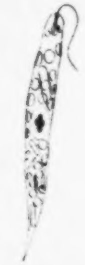
d Taxonomy of the group is involved.

e D. sertularia may clog filters.

f D. divergens may cause a fishy odor.

Green and Other Pigmented Flagellates

(fig 1 - 13 from Lackey and Callaway)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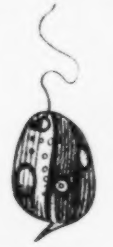
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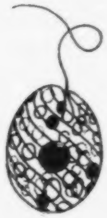
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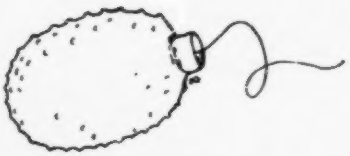
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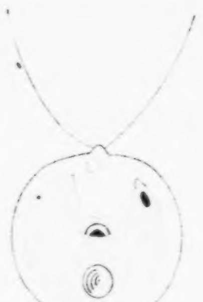
GREEN EUGLENOIDS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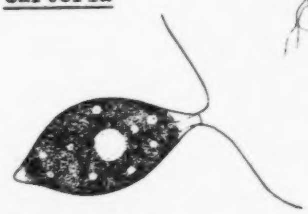
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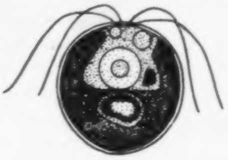
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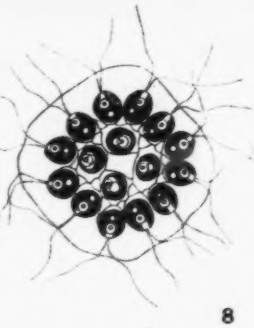
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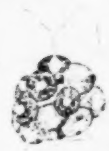
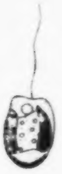

9 Pandorina

GREEN PHYTOMONADS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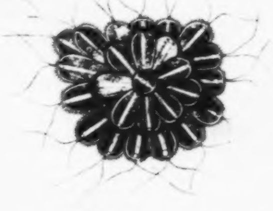
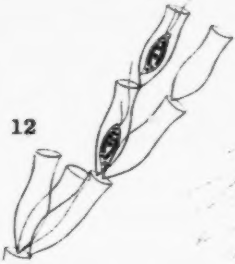
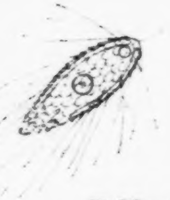

11 Synura


12 Dinobryon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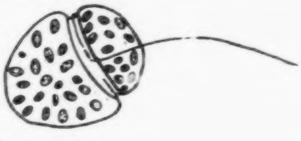
YELLOW CHRYSOMONADS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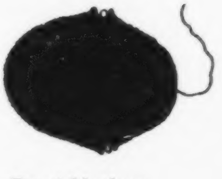
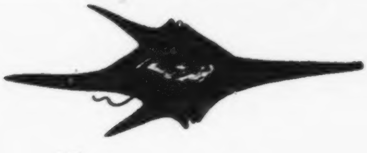

15 Peridinium


16 Ceratium

YELLOW-BROWN DINOFLAGELLATES

Ams 3-70

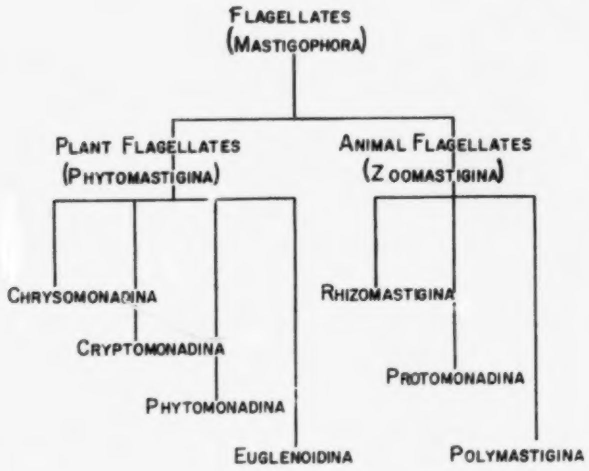

FLAGELLATES
(MASTIGOPHORA)
PLANT FLAGELLATES
(PHYTOMASTIGINA)
ANIMAL FLAGELLATES
(ZOOMASTIGINA)
CHRYSOMONADINA
CRYPTOMONADINA
PHYTOMONADINA
EUGLENOIDINA
RHIZOMASTIGINA
PROTOMONADINA
POLYMASTIGINA

Figure 17 Phylogenetic Family Tree of the Flagellates
(from Calaway and Lackey)

VII There are two distinctive groups whose systematic position is uncertain, the chloromonads and the cryptomonads. Only one genus of the latter group is included here.

- A Rhodomonas may range from bright red through pale brown to olive green.
  - 1 Cells compressed, narrow at the posterior end
  - 2 Two flagella of unequal length
  - 3 R. lacustris a small form intolerant of pollution

REFERENCES

1 Calaway, Wilson T. and Lackey, James B. Waste Treatment Protozoa Flagellata. Series No. 3. Univ. Fla. 140 pp. 1962.

2 Gojdics, M. The Genus Euglena. Univ. of Wisconsin Press, Madison. 1953.

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, MDS, Water Programs Operations, EPA, Cincinnati, OH 45268.

FILAMENTOUS GREEN ALGAE [1]

I MANY OF THESE FORMS ARE VISIBLE TO THE UNAIDED EYE

A They may be several inches or even a foot or more in length. In many cases they are not found as isolated filaments but develop in large aggregations to form floating or attached mats or tufts. The attached forms are generally capable of remaining alive after being broken away from the substrate.

B Included in the group are some of the most common and most conspicuous algae in freshwater habitats. A few of them have been given common names such as pond silk, green felt, frog-spawn algae, and stoneworts.

II CHARACTERISTICS OF FILAMENTOUS ALGAE

A These algae are in the form of cylindrical cells held together as a thread ("filament"), which may be in large clusters or growing separately. Some are attached to rocks or other materials while others are free. They may be unbranched ("simple") or branched; the tips are gradually narrowed ("attenuated") to a point. Some are surrounded by a mucilaginous envelope.

B Each cell is a short or long cylinder with a distinct wall. The protoplast contains a nucleus which is generally inconspicuous.

1 The plastid or chloroplast is the prominent structure. It contains chlorophyll and starch centers ("pyrenoids"), and varies in size, shape, and number per cell. It may be pressed against the wall ("parietal") or extend through the central axis of the cell ("axial").

2 Clear areas of cell sap ("vacuoles") are generally present in the cell.

C Specialized structures are present in some filaments.

1 Some filaments break up into "H" sections.

2 Apical caps are present in others.

3 Replicate end walls are present in some.

4 Some filaments are overgrown with a cortex.

5 Attached filaments have the basal cell developed into a "hold fast cell" (hapteron).

III REPRODUCTION MAY TAKE PLACE BY SEVERAL METHODS

A Cell division may occur in all cells or in certain selected ones.

B Spores called akinetes may be formed.

C Zoospores (motile) and aplanospores (non-motile) are common.

D Fragmentation of filaments may occur.

E Many kinds reproduce sexually, often with specialized gamete forming cells.

IV EXAMPLES OF FILAMENTOUS GREEN ALGAE ARE:

A Unbranched forms

*Spirogyra
*Mougeotia
Zygnema
Ulothrix
Microspora
Tribonema
Desmidium
Oedogonium

---

[1] Including a few yellow-brown and red algae.

*Planktonic or occasionally planktonic

B Branched forms

Cladophora
Pithopora
Stigeoclonium
Chaetophora
Draparnaldia
Rhizoclonium
Audouinella
Bulbochaete
Nitella

C Specialized and related forms

Schizomeris
Comsopogon
Batrachospermum
Chara
Lemanea
Vaucheria

V Habitats include the planktonic growths as well as surface mats or blankets and benthic attached forms on rocks in riffles of streams, at the shoreline of lakes and reservoirs, concrete walls, etc.

A Attached forms may break loose to become mixed with plankton or to form floating mats.

B Cladophora mats are a nuisance on many beaches on the Great Lakes.

VI IMPORTANCE OF FILAMENTOUS GREEN ALGAE

A They may cause clogging of sand filters, intake screens, and canals.

B They may produce tastes and odors in water or putrid odor (also producing H_2S which damage painted surfaces) when washed ashore around lakes and reservoirs.

C They may cause unsightly growths or interfere with fishing and swimming in recreation areas.

D Some are useful as indicators of water quality in relation to pollution.

E Together with other algae, they release oxygen required by fish, and for self-purification of streams.

F They may produce a slime which interferes with some industrial uses of water such as in paper manufacture and in cooling towers.

VII CLASSIFICATION

A Ulotrichaceae

Ulothrix, Microspora, Hormidium

B Cladophoraceae

Cladophora, Pithophora, Rhizoclonium

C Chaetophoraceae

Chaetophora, Stigeoclonium, Draparnaldia

D Oedogeniaceae

Oedogonium, Bulbochaete

E Schizomeridaceae

1 Schizomeris

F Ulvaceae

Enteromorpha, Monostroma

G Zygnemataceae

Zygnema, Spirogyra, Mougeotia

H Desmidiaceae

Desmidium, Hyalotheca

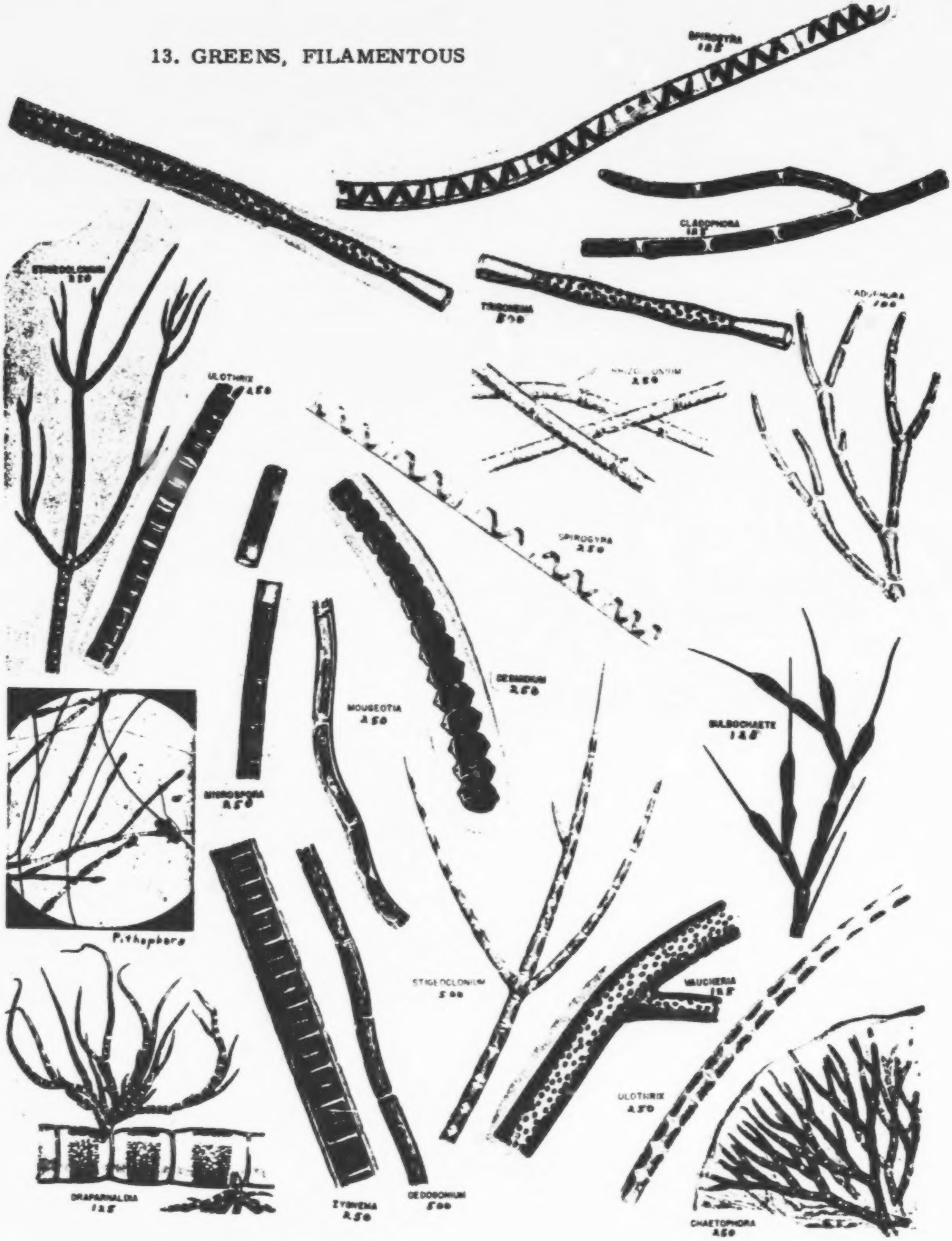
I Tribonemataceae

Tribonema, Bumilleria

J Characeae

Chara, Nitella, Tolypella

13. GREENS, FILAMENTOUS


SPIROGYRA
125
CLADOPHORA
125
TRIBONEMA
500
STIGEOCLONIUM
250
ULOTHRIX
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RHIZOCLONIUM
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CLADOPHORA
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SPIROGYRA
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DESMIDIUM
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MOUGEOTIA
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MICROSPORA
250
BULBOCHAETE
125
Pithophora
STIGEOCLONIUM
500
VAUCHERIA
125
ULOTHRIX
250
DRAPARNALDIA
125
ZYGNEMA
250
OEDOGONIUM
500
CHAETOPHORA
250

VIII IDENTIFICATION

A Branching and attenuation are of primary importance.

B Plastids: shape, location and number per cell are essential.

C Other characteristics include grouping of filaments, gelatinous envelope and special features such as "H" shaped fragments.

REFERENCES

1 Collins, F.S. 1909. The green algae of North America. Tufts College Studies, Scientific Series 2:79-480. Reprinted Hafner Publ. Co., 1928 (Reprinted, 1968) Lew's Books, San Francisco.

2 Faridi, M. A monograph of the freshwater species of Cladophora and Rhizoclonium. Ph.D. Thesis. University Microfilms, Ann Arbor.

3 Hirn, K.E. Monograph of the Oedogoniaceae. Hafner Publ., New York. 1960.

4 Pal, B.P., Kundu, B.C., Sundaralingam, V.S., and Venkataraman, G.S. Charophyta. Indian Coun. Agric. Res., New Delhi. 1962.

5 Soderstrom, J. Studies in Cladophora. Almquist, Uppsala. 1963.

6 Tilden, J. The Myxophyceae of North America. Minn. Geol. Surv. (Reprinted 1967, J. Cramer, Lehre, Germany) 1910.

7 Transeau, E.N. The Zygnemataceae. Ohio State Univ. Press. 1951.

8 Van der Hoek, C. Revision of the European species of Cladophora. Brill Publ, Leiden, Netherlands. 1963.

9 Wood, R.D. and Imahari, K. A revision of the Characeae. Volume I. Monograph (by Wood). Vol. II, Iconograph (by Wood & Imahari). 1964.

This outline was prepared by C.M. Palmer, Former Aquatic Biologist, In Charge, Interference Organisms Studies, Microbiology Activities, Research and Development, Cincinnati Water Research Laboratory, FWPCA.

COCCOID GREEN ALGAE[1]

I INTRODUCTION

For the sake of convenience, the non-motile green algae are to be discussed in two sections: those that tend to live as relatively discrete or free floating planktonic units, and those that tend to grow in masses or mats of material, often filamentous in nature, attached or free floating.

II The green or "grass green" algae is one of the most varied and conspicuous groups with which we have to deal. The forms mentioned below have been artificially grouped for convenience according to cell shape. Botanists would list these genera in several different categories in the family "Chlorophyceae."

These algae typically have a relatively high chlorophyll content, and the food reserves accumulated are typically starch. Thus these forms will usually give a typical black or deep purple color when treated with iodine.

A Individual cells of the following genera are perfectly round, or nearly so. The first does not form organized colonies. In the next two the colonies themselves tend to be round, and in the last, the colonies are triangular or irregular, and the cells bear long slender spines.

1 Chlorella cells are small and spherical to broadly elliptical. They have a single parietal chloroplast. This is a very large genus with an unknown number of similar appearing species, living in a great variety of habitats. Although often accumulating in great numbers, organized colonies are not formed.

a Chlorella ellipsoides is reported to be a common plankton form.

b Chlorella pyrenoidosa and Chlorella vulgaris are often found in organically enriched waters. Indeed a dominance of Chlorella species is considered in some places to be an indication that a sewage stabilization pond is functioning to maximum capacity.

c Chlorella pyrenoidosa is reported as a filter clogger in water treatment plants.

2 Sphaerocystis colonies are free floating and almost always with a perfectly spherical, homogeneous gelatinous envelope. Up to 32 spherical cells may be included. Sphaerocystis scheoeteri, the only species, is of wide occurrence in the plankton of lakes and reservoirs.

3 Coelastrum forms coenobial* colonies of up to 128 cells. Generally spherical or polygonal in shape--both cells and colony. Cells connected by protoplasmic processes of varying length. Coelastrum microporum is often reported in the plankton of water supplies. Not surrounded by gelatinous envelope as in Sphaerocystis.

4 Micractinium. The cells of this alga are spherical to broadly ellipsoidal and are usually united in irregular 4-celled coenobes. These in turn are almost always united with other coenobes to form multiple associations of up to 100 or more cells. The free face of

---

[1] Including miscellaneous yellow-brown algae.

*A coenobe is a colony in which the number of cells does not increase during the life of the colony. It was established by the union of several independent swimming cells which simply stick together and increase in size.

each cell in a coenobe bears from one to seven very long slender setae or hairs.

Micractinium pusillum. This is a strictly planktonic genus.

B Individual cells of the following genera are elongate. In the first two they are relatively straight or irregular and pointed. The next two are also long and pointed, but bent into a tight "C" shape (one in a gelatinous envelope, one naked). The last one (*Actinastrum*) is long and straight, but with blunt ends, and with the cells of a coenobe attached at a point.

1 *Ankistrodesmus* cells are usually long and slender, tapering to sharp point at both ends. They may be straight, curved, or twisted into loose aggregations. *Ankistrodesmus falcatus* is often found in the plankton in water supplies and is considered to be one of the forms indicative of clean water.

2 *Schroederia* is a solitary, free floating alga. Cells are long and pointed at both ends. May be bent in various ways. Terminal points are continued as long slender spines which may be forked and bent back, or end as a plate. Of the three species reported in this country, *Schroederia setigera* has been reported in water supplies.

3 *Selenastrum* cells are pointed at both ends, and bent so that their tips approach each other. They tend to occur in groups of 4, 8, or 16, which may be associated with other groups to form masses of a hundred or more cells. There is no gelatinous envelope. *Selenastrum gracile* occurs in the plankton of water supplies.

4 *Kirchneriella*. The cells of this genus are generally relatively broad, tapering to a sharp or rounded point at each end, and the whole cell bent into a C-shape. They usually occur in groups of four to eight in a broad, homogeneous, gelatinous matrix. *Kirchneriella lunaris* is known principally from the plankton.

5 *Actinastrum colonies* or "coenobes" are composed of 4, 8, or 16 elongate cells that radiate in all directions from a common center.

Actinastrum is a widely distributed plankton organism. There are two species:

Actinastrum gracillimum and *Actinastrum Hantzschii* differ only in the sharpness of the taper toward the tips of the cells. The former has relatively little taper, and the latter, more.

C Cells of the following genera are associated in simple naked colonies. The first has elongate cells arranged with their long axes parallel (although some cells may be curved). The last two are flat plate-like coenobes. *Crucigenia* has four-celled coenobes while *Pediastrum* coenobes may be larger, appear plate-like, and are much more ornate.

1 *Scenedesmus* is a flat plate of elliptical to double ended pointed cells arranged with their long axes parallel. Coenobes consist of up to 32, but usually 4 to 8 cells. The number of cells in a coenobe may vary from mother to daughter colony. The appearance of cells may vary considerably with the species.

a *Scenedesmus bijuga*, *S. dimorphus*, and *S. quadricauda* are common planktonic forms.

b *Scenedesmus quadricauda* is also common in organically enriched water, and may become dominant.

c *Scenedemus abundans* is reported to impart a grassy odor to drinking water.

2 Crucigenia forms free floating four-celled coenobes that are solitary or joined to one another to form plate-like multiple coenobes of 16 or more cells. The cells may be elliptical, triangular, trapezoidal, or semi-circular in surface view. Crucigenia quadrata is a species often reported from water supplies.

3 Pediastrum. Colonies are free floating with up to 128 polygonal cells arranged in a single plane. There may or may not be open spaces between the cells. The exact arrangement of the cells seems to depend largely on the chance distribution of the original motile swarming zoaspores at the time the coenobe was formed. Peripheral cells may differ in shape from interior cells.

a Pediastrum boryanum and P. duplex are frequently found in the plankton, but seldom dominate.

b Pediastrum tetras has been reported to impart a grassy odor to water supplies.

D Cells of the following Genera are slightly elongated.

1 Oocystis. The cells of Oocystis may be solitary, or up to 16 cells may be surrounded by a partially gellatinized and greatly expanded mother cell wall. Cells may be ellipsoidal or almost cylindrical, cell wall thin, no spines or other ornamentation. Oocystis borgei, for example, is of frequent occurrence in the plankton.

2 Dimorphococcus cells are arranged in groups of four, and these tetrads are united to one another in irregularly shaped free floating colonies by the branching remains of old mother-cell walls. Two shapes of cell are normally found in each tetrad (hence the name), two longer ovate cells end to end, and a pair of slightly shorter, C-shaped cells on either side. Dimorphococcus lunatus is a widely distributed plankton organism, sometimes reported in considerable numbers.

E A distinctive group of green algae characterized by a median constriction dividing the cell into two geometrically similar halves is known generally as the "desmids." (Closterium and Penium do not have this construction). Each half of the cell is known as a "semicell." The nucleus lies in the "isthmus." Extremes of ornamentation and structural variety exist. Most are unicellular, but a few are filamentous or have the cells associated in shapeless colonies. They are found sparingly in the plankton almost everywhere, but predominate in acid waters.

1 Closterium is one of the exceptional genera without a median constriction. The cells are elongate, attenuated toward the tips but not sharply pointed, usually somewhat bent.

a Closterium aciculare is a planktonic species.

b Closterium moniliforme is reported as a filter clogging organism.

2 Cosmarium is a large, poorly defined genus of over 280 species, many of which apparently intergrade with other genera such as Staurastrum. In general, it can be said that Cosmarium species are relatively small, with a length only slightly greater than the width, and with a deep median constriction. Shapes of the semicells may vary greatly. Although shallow surface ornamentation may occur, long spines do not occur.

a Cosmarium botrytis is reported in plankton from water supply reservoirs.

b Cosmarium portianum is said to impart a grassy odor to water.

c Other species have been reported to be sufficiently resistant to chlorine to penetrate rapid sand filters and occur in distribution systems in considerable numbers.

3 Micrasterias is relatively common, ornate.

4 Euastrum cells tend to be at least twice as long as broad, with a deeply constricted isthmus, and a dip or incision at the tip of each semicell. The cell wall may be smooth, granulate, or spined.

Euastrum oblongum is reported as a planktonic species from water reservoirs. It has also been noted as intolerant of pollution, and hence an indicator of clean water.

5 Staurastrum is the commonest of the desmids in the plankton of fresh waters; the genus contains upwards of 245 species in the United States alone. Intergradation with other genera such as Cosmarium make it a difficult group to define. Most of the species are radially symmetrical, and almost all have a deeply constricted isthmus. The cell wall may be smooth, ornamented, or spined in a variety of ways. Relatively long truncated processes extending from the cell body in symmetrical patterns are common.

a Staurastrum polymorphum is a typical planktonic form.

b Staurastrum punctulatum is reported as an indicator of clean water.

c Staurastrum paradoxicum causes a grassy odor in water.

III A type of "green" alga known as "golden green" (Xanthophyceae) is represented in the plankton by two genera. In these algae there is a predominance of yellow over green pigments, hence frequently imparting a yellowish or golden tint to the cell. Reserve food material is stored as oil and leucosin, rather than as starch, hence giving a negative test with iodine in most cases.

A Botryococcus braunii is a widely distributed plankton alga, though it is rarely abundant.

1 The plant body is a free floating colony of indefinite shape, with a cartilaginous and hyaline or orange-colored envelope; surface greatly wrinkled and folded.

2 Individual cells lie close together, in several aggregates connected in reticular fashion by strands of the colonial envelope.

3 The envelope structure tends to obscure cell structure. Considerable deep orange colored oil may collect within the envelope, outside of the cells, obscuring cell structure.

B Ophiocytium capitatum like Botryococcus, is widely distributed, but seldom abundant.

1 Both ends of cylindrical cell are rounded, with a sharp spine extending therefrom.

2 Many nuclei and several chloroplasts are present.

REFERENCES

1 Palmer, C.M. Algae in Water Supplies. Government Printing Office. PHS Publication No. 657. 1959.

2 Smith, G.S. Phytoplankton of the Inland Lakes of Wisconsin. Part I. Bulletin No. 57, Scientific Series No. 12. 1920.

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DIATOMS

I GENERAL CHARACTERISTICS

A Diatoms have cells of very rigid form due to the presence of silica in the wall. They contain a brown pigment in addition to the chlorophyll. Their walls are ornamented with markings which have a specific pattern for each kind.

1 The cells often are isolated but others are in filaments or other shapes of colonies.

2 The protoplast contains normal cell parts, the most conspicuous being the plastids. No starch is present.

B Cell shapes include the elongate ("pennate") and the short cylindric ("centric") one view of which is circular.

1 Pennate diatoms may be symmetrical, transversely unsymetrical, or longitudinally unsymmetrical.

C Wall is formed like a box with a flanged cover fitting over it.

1 "Valve" view is that of the top of the cover or the bottom of the box.

2 "Girdle" view is that of the side where flange of cover fits over the box.

3 End view is also possible for pennate types.

D Cell markings include:

1 Raphe or false raphe extending longitudinally.

2 Striations which are lines of pores extending from the area of the raphe to the margin. Coarse ones are "costae".

3 Nodules which may be terminal and central.

4 Internal shelves ("septae") extending longitudinally or transversely.

II REPRODUCTION

A The common method is by cell division. Two new half cells are formed between the halves of the parent cell.

B Auxospores and gametes may also be formed.

III EXAMPLES OF COMMON DIATOMS:

A Pennate, symmetrical:

Navicula
Pinnularia
Synedra
Nitzschia
Diatoma
Fragilaria
Tabellaria
Cocconeis

B Pennate, unsymmetrical:

Gomphonema
Surirella
Cymbella
Achnanthes
Asterionella
Meridion

C Centric:

Cyclotella
Stephanodiscus
Melosira

IV Habitats include fresh and salt water. Both planktonic and attached forms occur, the latter often are broken loose. They may be attached by stalks or by their slimy surface.

A Many diatoms are more abundant in late autumn, winter, and early spring than in the warmer season.

B The walls of dead diatoms generally remain undecomposed and may be common in water. Many deposits of fossil diatoms exist.

V Importance of diatoms is in part due to their great abundance and their rigid walls.

A They are the most important group of organisms causing clogging of sand filters.

B Several produce tastes and odors in water, including the obnoxious fishy flavor.

C Mats of growth may cause floors or steps of swimming pools to be slippery.

D They may be significant in determining water quality in relation to pollution.

E They release oxygen into the water.

VI Classification. There are several thousand species of diatoms. Only the most common of the freshwater forms are considered here.

A Centrales Group

1 Representative genera:

Cyclotella
Stephanodiscus
Melosira
Rhizosolenia
Biddulphia

B Pennals Group

1 Fragilarineae. The false raphe group.

Representative genera:

Tabellaria
Meridion
Diatoma
Fragilaria
Synedra
Asterionella

2 Achnanthineae. Group with cells having one false and one true raphe.

a Representative genera:

Cocconeis
Achnanthes

3 Naviculineae. True raphe group with raphe in center of valve.

a Representative genera:

Navicula
Pinnularia
Stauroneis
Pleurosigma
Amphiprora
Gomphonema
Cymbella
Epithemia

4 Surirellineae. True raphe group with raphe near one side of valve.

a Representative genera:

Nitzschia
Cymatopleura
Surirella
Campylodiscus

VII IDENTIFICATION OF DIATOMS

A Some genera are easily recognized by their distinctive shape.

B Many genera and most species can be determined only after diatoms are freed of their contents and observed under the high magnification of an oil immersion lens of the compound microscope.

C Contents of the cell are generally not used in identification. Only the characteristics of the wall are used.

D For identification of genera, most important features include:

  1 Cell shape, and form of colony

  2 Raphe and false raphe

  3 Striations

  4 Septa

E For identification of species, measurements involving the number of striae per 10 microns, the direction of the striae and many other characteristics may be needed.

REFERENCES

1 Boyer, C.S. The Diatomaceae of Philadelphia and Vicinity. J.B. Lippincott Co. Philadelphia. 1916. p 143.

2 Boyer, C.S. Synopsis of North America Diatomaceae. Parts I (1927) and II (1928). Proceedings of the Academy of Natural Sciences. Philadelphia.

3 Elmore, C.J. The Diatoms of Nebraska. University of Nebraska Studies. 21: 22-215. 1921.

4 Hohn, M.H. A Study of the Distribution of Diatoms in Western New York State. Cornell University Agricultural Experimental Station. Memoir 308. pp 1-39. 1951.

5 Pascher, A. Bacillariophyta (Diatomeae). Heft 10 in Die Susswasser-Flora Mitteleuropas, Jena. 1930. p 466.

6 Patrick, R. A Taxonomic and Ecological Study of Some Diatoms from the Pocono Plateau and Adjacent Regions. Farlowia. 2:143-221. 1945.

7 Patrick, Ruth and Reimer, Charles W. The Diatoms of the United States. Vol. 1 Fragilariaceae, Eunotiaceae, Achnanthaceae, Naviculaceae. Monog. 13. Acad. Nat. Sci. Philadelphia. 688 pp. 1966.

8 Smith, G.M. Class Bacillariophyceae. Freshwater Algae of the United States. pp 440-510, 2nd Edition. McGraw Hill Book Co. New York. 1950.

9 Tiffany, L.H. and Britton, M.E. Class Bacillariophyceae. The Algae of Illinois. pp 214-296. University of Chicago Press. 1952.

10 Ward, H.B. and Whipple, G.C. Class I, Bacillariaceae (Diatoms). Freshwater Biology. pp 171-189. John Wiley & Sons. New York. 1948.

11 Weber, C. I. A Guide to the Common Diatoms at Water Pollution Surveillance System Stations. FWPCA. Cincinnati. 101 pp. 1966.

12 Whipple, G.C., Fair, G.M., and Whipple, M.C. Diatomaceae. Microscopy of Drinking Water. Chapter 21. 4th Edition. John Wiley & Sons. New York. 1948.

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FILAMENTOUS BACTERIA

I INTRODUCTION

There are a number of types of filamentous bacteria that occur in the aquatic environment. They include the sheathed sulfur and iron bacteria such as Beggiatoa, Crenothrix and Sphaerotilus, the actinomycetes which are unicellular microorganisms that form chains of cells with special branchings, and Gallionella, a unicellular organism that secretes a long twisted ribbon-like stalk. These filamentous forms have at times created serious problems in rivers, reservoirs, wells, and water distribution systems.

II BEGGIATOA

Beggiatoa is a sheathed bacterium that grows as a long filamentous form. The flexible filaments may be as large as 25 microns wide and 100 microns long. (Figure 1)

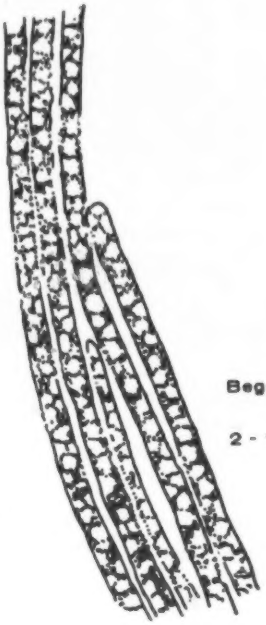

Figure 1

Transverse separations within the sheath indicate that a row of cells is included in one sheath. The sheath may be clearly visible or so slight that only special staining will indicate that it is present.

The organism grows as a white slimy or felted cover on the surface of various objects undergoing decomposition or on the surface of stagnant areas of a stream receiving sewage. It has also been observed on the base of a trickling filter and in contact aerators.

It is most commonly found in sulfur springs or polluted waters where H_2S is present. Beggiatoa is distinguished by its ability to deposit sulfur within its cells; the sulfur deposits appear as large refractile globules. (Figure 2)

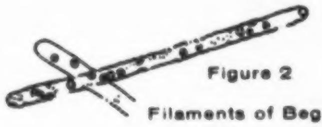

Figure 2

Filaments of Beggiatoa containing granules of sulphur.

When H_2S is no longer present in the environment, the sulfur deposits disappear. Dr. Pringsheim of Germany has recently proved that the organism can grow as a true autotroph obtaining all its energy from the oxidation of H_2S and using this energy to fix CO_2 into all material. It can also use certain organic materials if they are present along with the H_2S.

Faust and Wolfe, and Scotten and Stokes have grown the organism in pure culture in this country. Beggiatoa exhibits a motility that is quite different from the typical flagellated motility of most bacteria; the filaments have a flexible gliding motion.

The only major nuisance effect of Beggiatoa known has been overgrowth on trickling filters receiving waste waters rich in H_2S. The normal microflora of the filter was suppressed and the filter failed to give good treatment. Removal of the H_2S from the water by blowing air through the water before it reached the filters caused the slow decline of the Beggiatoa and a recovery of the normal microflora. Beggiatoa usually indicates polluted conditions with the presence of H_2S rather than being a direct nuisance.

III ACTINOMYCETES AND EARTHY ODORS IN WATER

Actinomycetes are unicellular microorganisms, 1 micron in diameter, filamentous, non-sheathed, branching monopodially, and reproduced by fission or by means of special conidia. (Figure 3)

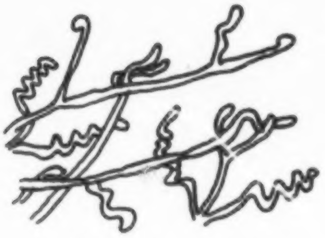

Figure 3 Filaments of Actinomycetes

Their filamentous habit and method of sporulation is reminiscent of fungi. However, their size, chemical composition, and other characteristics are more similar to bacteria. (Figure 4)

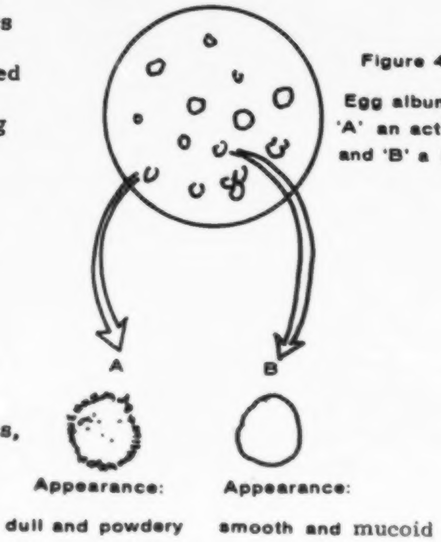

Figure 4

Egg albumin isolation plate. 'A' an actinomycete colony, and 'B' a bacterial colony

These organisms may be considered as a group intermediate between the fungi and the bacteria. They require organic matter for growth but can use a wide variety of substances and are widely distributed.

Actinomycetes have been implicated as the cause of earthy odors in some drinking waters (Romano and Safferman, Silvey and Roach) and in earthy smelling substance has been isolated from several members of the group by Gerber and Lechevalier. Safferman and Morris have reported on a method for the "Isolation and Enumeration of Actinomycetes Related to Water Supplies." But the actinomycetes are primarily soil microorganisms and often grow in fields or on the banks of a river or lake used for the water supply. Although residual chlorination will kill the organisms in the treatment plant or distribution system, the odors often are present before the water enters the plant. Use of permanganate oxidation and activated carbon filters have been most successful of the methods tried to remove the odors from the water. Control procedures to prevent the odorous material from being washed into the water supply by rains or to prevent possible development of the actinomycetes in water rich in decaying organic matter is still needed.

IV FILAMENTOUS IRON BACTERIA

The filamentous iron bacteria of the Sphaerotilus-Leptothrix group, Crenothrix, and Gallionella have the ability to either oxidize manganous or ferrous ions to manganic or ferric salts or are able to accumulate precipitates of these compounds within the sheaths of the organisms. Extensive growths or accumulations of the empty, metallic encrusted sheaths devoid of cells, have created much trouble in wells or water distribution systems. Pumps and back surge valves have been clogged with masses of material, taste and odor problems have occurred, and rust colored masses of material have spoiled products in contact with water.

Crenothrix polyspora has only been examined under the microscope as we have never been able to grow it in the laboratory. The organism is easily recognized by its special morphology. Dr. Wolfe of the University of Illinois has published photomicrographs of the organism. (Figure 5)

Organisms of the Sphaerotilus-Leptothrix group have been extensively studied by many investigators (Dondero et. al., Dondero, Stokes, Waitz and Lackey, Mulder and van Veen, and Amberg and Cormack.) Under different environmental conditions the morphological appearance of the organism varies. The usual form found in polluted streams or bulked activated sludge is Sphaerotilus natans. (Figure 6)

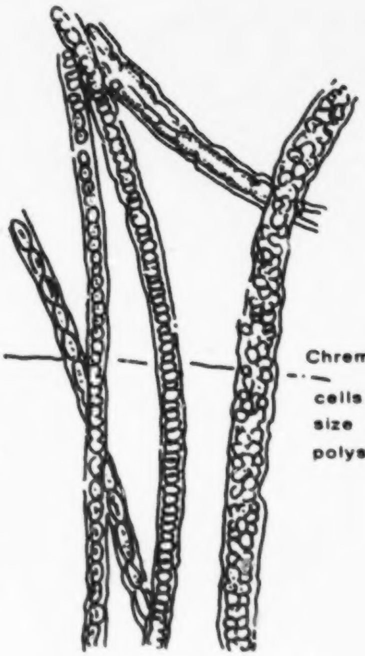

Figure 5

Chremothrix polyspora

cells are very variable in size from small cocci or polyspores to cells 3×12µ

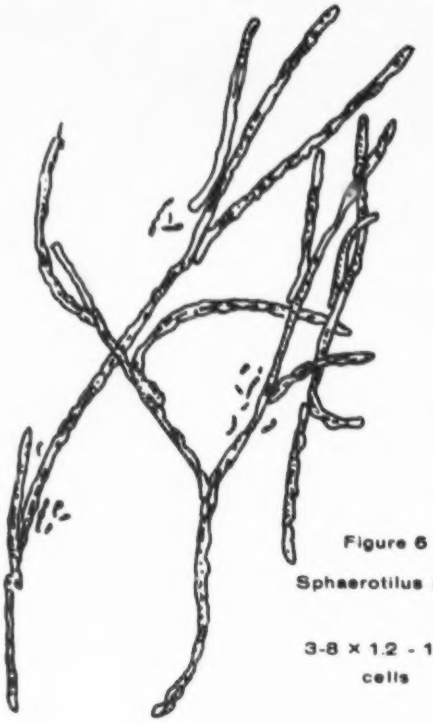

Figure 6

Sphaerotilus natans

3-8 × 1.2 - 1.8µ cells

This is a sheathed bacterium consisting of long, unbranched filaments, whereby individual rod-shaped bacterial cells are enclosed in a linear order within the sheath. The individual cells are 3-8 microns long and 1.2-1.8 microns wide. Sphaerotilus grows in great masses; at times in streams or rivers that receive wastes from pulp mills, sugar refineries, distilleries, slaughterhouses, or milk processing plants. In these conditions, it appears as large masses or tufts attached to rocks, twigs, or other projections and the masses may vary in color from light grey to reddish brown. In some rivers large masses of Sphaerotilus break loose and clog water intake pipes or foul fishing nets. When the cells die, taste and odor problems may also occur in the water.

Amberg, Cormack, and Rivers and McKeown have reported on methods to try to limit the development of Sphaerotilus in rivers by intermittant discharge of wastes. Adequate control will probably only be achieved once the wastes are treated before discharge to such an extent that the growth of Sphaerotilus is no longer favored in the river. Sphaerotilus grows well at cool temperatures and slightly low DO levels in streams receiving these wastes and domestic sewage. Growth is slow where the only nitrogen present is inorganic nitrogen; peptones and proteins are utilized preferentially.

Gallionella is an iron bacterium which appears as a kidney-shaped cell with a twisted ribbon-like stalk emanating from the concavity of the cell. Gallionella obtains its energy by oxidizing ferrous iron to ferric iron and uses only CO_2 and inorganic salts to form all of the cell material; it is an autotroph. Large masses of Gallionella may cause problems in wells or accumulate in low-flow low-pressure water mains. Super chlorination (up to 100 ppm of sodium hypochlorite for 48 hours) followed by flushing will often remove the masses of growth and periodic treatment will prevent the nuisance effects of the extensive masses of Gallionella. (Figure 7)

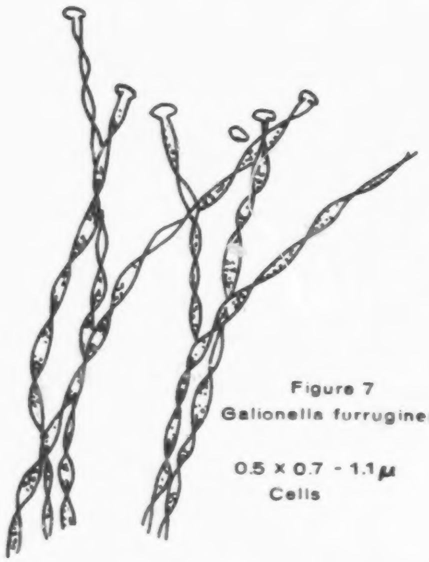

Figure 7
Galionella furruginea

0.5 x 0.7 - 1.1μ
Cells

REFERENCES

Beggiatoa

1 Faust, L. and Wolfe, R.S. Enrichment and Cultivation of Beggiatoa Alba. Jour. Bact., 81:99-106. 1961.

2 Scotten, H. L. and Stokes, J. L. Isolation and Properties of Beggiatoa. Arch Fur. Microbiol. 42:353-368. 1962.

3 Kowallik, U. and Pringsheim, E.G. The Oxidation of Hydrogen Sulfide by Beggiatoa. Amer. Jour. of Botany. 53:801-805. 1966.

Actinomycetes and Earthy Odors

4 Silvey, J.K.G. et al. Actinomycetes and Common Tastes and Odors. JAWWA, 42:1018-1026. 1950.

5 Safferman, R.S. and Morris, M.E. A Method for the Isolation and Enumeration of Actinomycetes Related to Water Supplies. Robert A. Taft Sanitary Engineering Center Tech. Report W-62-10. 1962.

6 Gerber, N.N. and Lechevalier, H.A. Geosmin, an Earthy-Smelling Substance Isolated from Actinomycetes. Appl. Microbiol. 13:935-938. 1965.

Filamentous Iron Bacteria

7 Wolfe, R.S. Cultivation, Morphology, and Classification of the Iron Bacteria. JAWWA. 50:1241-1249. 1958.

8 Kucera, S. and Wolfe, R.S. A Selective Enrichment Method for Gallionella ferruginea. Jour. Bacteriol. 74:344-349. 1957.

9 Wolfe, R.S. Observations and Studies of Crenothrix polyspora. JAWWA, 52:915-918. 1960.

10 Wolfe, R.S. Microbiol. Concentration of Iron and Manganese in Water with Low Concentrations of these Elements. JAWWA. 52:1335-1337. 1960.

11 Stokes, J.L. Studies on the Filamentous Sheathed Iron Bacterium Sphaerotilus natans. Jour. Bacteriol. 67:278-291. 1954.

12 Waitz, S. and Lackey, J.B. Morphological and Biochemical Studies on the Organism Sphaerotilus natans. Quart. Jour. Fla. Acad. Sci. 21(4):335-340. 1958.

13 Dondero, N.C., Philips, R.A. and Henkelkian, H. Isolation and Preservation of Cultures of Sphaerotilus. Appl. Microbiol. 9:219-227. 1961.

14 Dondero, N.C. Sphaerotilus, Its Nature and Economic Significance. Advances Appl. Microbiol. 3:77-107. 1961.

15 Mulder, E.G. and van Veen, W.L. Investigations on the Sphaerotilus-Leptothrix Group. Antonie van Leewenhoek 29:121-153. 1963.

16 Amberg, H.R. and Cormack, J.F. Factors Affecting Slime Growth in the Lower Columbia River and Evaluation of Some Possible Control Measures. Pulp and Paper Mag. of Canada. 61:T70-T80. 1960.

17 Amberg, H.R., Cormack, J.F. and Rivers, M.R. Slime Growth Control by Intermittant Discharge of Spent Sulfite Liquor. Tappi. 45:770-779. 1962.

18 McKeown, J.J. The Control of Sphaerotilus natans. Ind. Water and Wastes. 8:(3) 19-22 and 8:(4)30-33. 1963.

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FUNGI AND THE "SEWAGE FUNGUS" COMMUNITY

I INTRODUCTION

A Description

Fungi are heterotrophic achylorophyllous plant-like organisms which possess true nuclei with nuclear membranes and nucleoli. Dependent upon the species and in some instances the environmental conditions, the body of the fungus, the thallus, varies from a microscopic single cell to an extensive plasmodium or mycelium. Numerous forms produce macroscopic fruiting bodies.

B Life Cycle

The life cycles of fungi vary from simple to complex and may include sexual and asexual stages with varying spore types as the reproductive units.

C Classification

Traditionally, true fungi are classified within the Division Eumycotina of the Phylum Mycota of the plant kingdom. Some authorities consider the fungi an essentially monophyletic group distinct from the classical plant and animal kingdoms.

II ACTIVITY

In general, fungi possess broad enzymatic capacities. Various species are able to actively degrade such compounds as complex polysaccharides (e.g., cellulose, chitin, and glycogen), proteins (casein, albumin, keratin), hydrocarbons (kerosene) and pesticides. Most species possess an oxidative or microaerophilic metabolism, but anaerobic catabolism is not uncommon. A few species show anaerobic metabolism and growth.

III ECOLOGY

A Distribution

Fungi are ubiquitous in nature and members of all classes may occur in large numbers in aquatic habitats. Sparrow (1968) has briefly reviewed the ecology of fungi in freshwaters with particular emphasis on the zoosporic phycomycetes. The occurrence and ecology of fungi in marine and estuarine waters has been examined recently by a number of investigators (Johnson and Sparrow, 1961; Johnson, 1968; Myers, 1968; van Uden and Fell, 1968).

B Relation to Pollution

Wm. Bridge Cooke, in a series of investigations (Cooke, 1965), has established that fungi other than phycomycetes occur in high numbers in sewage and polluted waters. His reports on organic pollution of streams (Cooke, 1961; 1967) show that the variety of the Deuteromycete flora is decreased at the immediate sites of pollution, but dramatically increased downstream from these regions.

Yeasts, in particular, have been found in large numbers in organically enriched waters (Cooke, et al., 1960; Cooke and Matsuura, 1963; Cooke, 1965b; Ahearn, et al., 1968). Certain yeasts are of special interest due to their potential use as "indicator" organisms and their ability to degrade or utilize proteins, various hydrocarbons, straight and branch chained alkyl-benzene sulfonates, fats, metaphosphates, and wood sugars.

C "Sewage Fungus" Community (Plate I)

A few microorganisms have long been termed "sewage fungi." The most common microorganisms included in this group are the iron bacterium Sphaerotilus natans and the phycomycete Leptomitus lacteus.

1 Sphaerotilus natans is not a fungus; rather it is a sheath bacterium of the order chlamydobacteriales. This polymorphic bacterium occurs commonly in organically enriched streams where it may produce extensive slimes.

a Morphology

Characteristically, S. natans forms chains of rod shaped cells (1.1 - 2.0μ x 2.5 - 17μ) within a clear sheath or trichome composed of a protein-polysaccharidae-lipid complex. The rod cells are frequently motile upon release from the sheath; the flagella are lophotrichous. Occasionally two rows of cells may be present in a single sheath. Single trichomes may be several mm in length and bent at various angles. Empty sheaths, appearing like thin cellophane straws, may be present.

b Attached growths

The trichomes are cemented at one end to solid substrata such as stone or metal, and their cross attachment and bending gives a superficial similarity to true fungal hyphae. The ability to attach firmly to solid substrates gives S. natans a selective advantage in the population of flowing streams. For more thorough reviews of S. natans see Prigsheim (1949) and Stokes (1954).

2 Leptomitus lacteus also produces extensive slimes and fouling flocs in fresh waters. This species forms thalli typified by regular constrictions.

a Morphology

Cellulin plugs may be present near the constrictions and there may be numerous granules in the cytoplasm. The basal cell of the thallus may possess rhizoids.

b Reproduction

The segments delimited by the partial constrictions are converted basipetally to sporangia. The zoospores are diplanetic (i.e., dimorphic) and each possesses one whiplash and one tinsel flagellum. No sexual stage has been demonstrated for this species.

c Distribution

For further information on the distribution and systematics of L. lacteus see Sparrow (1960), Yerkes (1966) and Emerson and Weston (1967). Both S. natans and L. lacteus appear to thrive in organically enriched cold waters (5°-22°C) and both seem incapable of extensive growth at temperatures of about 30°C.

d Gross morphology

Their metabolism is oxidative and growth of both species may appear as reddish brown flocs or stringy slimes of 30 cm or more in length.

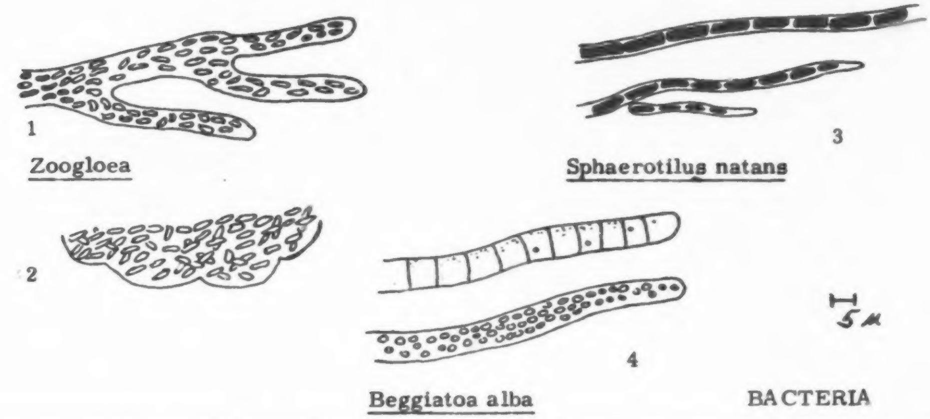
e Nutritive requirements

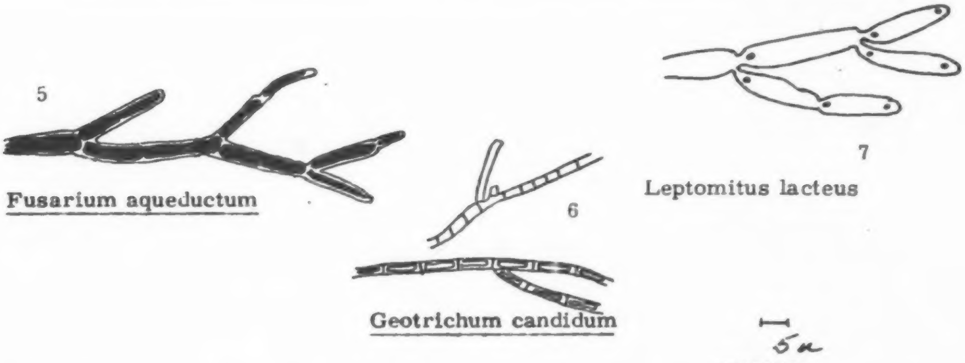
Sphaerotilus natans is able to utilize a wide variety of organic compounds, whereas L. lacteus does not assimilate simple

PLATE I

"SEWAGE FUNGUS" COMMUNITY OR "SLIME GROWTHS"

(Attached "filamentous" and slime growths)


1
Zoogloea
3
Sphaerotilus natans
2
4
Beggiatoa alba
5 µ
BACTERIA


5
Fusarium aqueductum
6
Geotrichum candidum
7
Leptomitus lacteus
5 µ
FUNGI

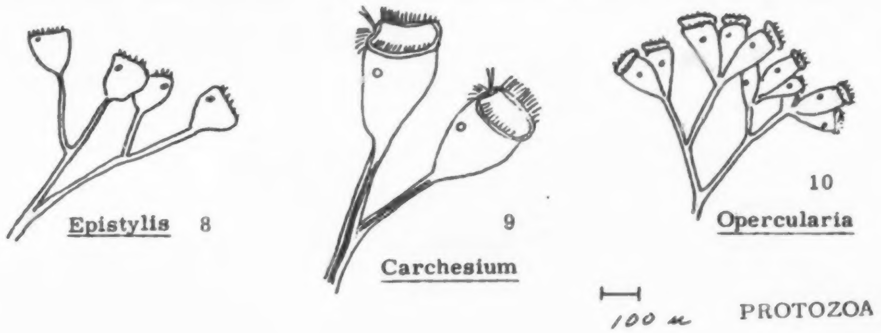

Epistylis 8
9
Carchesium
10
Opercularia
100 µ
PROTOZOA

PLATE II

REPRESENTATIVE FUNGI

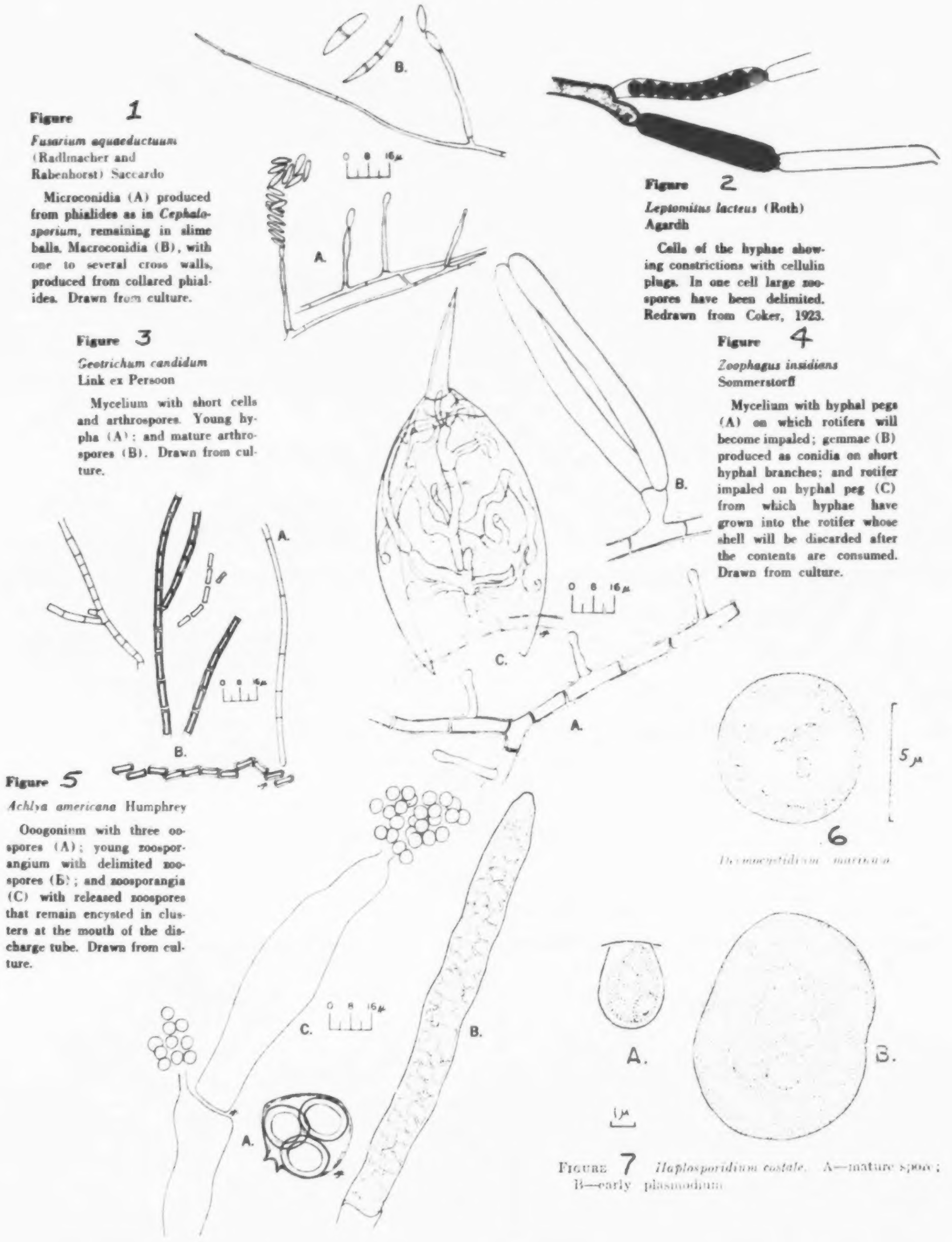


Figure 1

Fusarium aquaeductuum (Radlmacher and Rabenhorst) Saccardo

Microconidia (A) produced from phialides as in *Cephalosporium*, remaining in slime balls. Macroconidia (B), with one to several cross walls, produced from collared phialides. Drawn from culture.

Figure 2

Leptomitus lacteus (Roth) Agardh

Cells of the hyphae showing constrictions with cellulin plugs. In one cell large zoospores have been delimited. Redrawn from Coker, 1923.

Figure 3

Geotrichum candidum Link ex Persoon

Mycelium with short cells and arthrospores. Young hypha (A); and mature arthrospores (B). Drawn from culture.

Figure 4

Zoophagus insidians Sommerstorff

Mycelium with hyphal pegs (A) on which rotifers will become impaled; gemmae (B) produced as conidia on short hyphal branches; and rotifer impaled on hyphal peg (C) from which hyphae have grown into the rotifer whose shell will be discarded after the contents are consumed. Drawn from culture.

Figure 5

Achlya americana Humphrey

Oogonium with three oospores (A); young zoosporangium with delimited zoospores (B); and zoosporangia (C) with released zoospores that remain encysted in clusters at the mouth of the discharge tube. Drawn from culture.

6 *Dermocystidium marinum*

FIGURE 7 *Haplosporidium costale*. A—mature spore; B—early plasmodium

Figures 1 through 5 from Cooke; Figures 6 and 7 from Galtsoff.

sugars and grows most luxuriantly in the presence of organic nitrogenous wastes.

3 Ecological roles

Although the "sewage fungi" on occasion attain visually noticeable concentrations, the less obvious populations of deuteromycetes may be more important in the ecology of the aquatic habitat. Investigations of the past decade indicate that numerous fungi are of primary importance in the mineralization of organic wastes; the overall significance and exact roles of fungi in this process are yet to be established.

D Predacious Fungi

1 Zoophagus insidians

(Plate II, Figure 4) has been observed to impair functioning of laboratory activated sludge units (see Cooke and Ludzack).

2 Arthrobotrys is usually found along with Zoophagus in laboratory activated sludge units. This fungus is predacious upon nematodes. Loops rather than "pegs" are used in snaring nematodes.

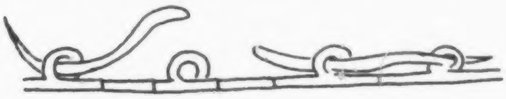


PLATE II (Figure 4)

IV CLASSIFICATION

In recent classification schemes, classes of fungi are distinguished primarily on the basis of the morphology of the sexual and zoosporic stages. In practical schematics, however, numerous fungi do not demonstrate these stages. Classification must therefore be based on the sum total of the morphological and/or physiological characteristics. The extensive review by Cooke (1963) on methods of isolation and classification of fungi from sewage and polluted waters precludes the need herein of extensive keys and species illustrations. A brief synopsis key of the fungi adapted in part from Alexopholous (1962) is presented on the following pages.

This outline was prepared by Dr. Donald G. Ahearn, Professor of Biology, Georgia State College, Atlanta, Georgia 30303.

KEY TO THE MAJOR TAXA OF FUNGI

1 Definite cell walls lacking, somatic phase a free living Plasmodium........................ .... Sub-phylum Myxomycotina ..(true slime molds)..Class *Myxomycetes*

1' Cell walls usually well defined, somatic phase not a free-living Plasmodium................ ....(true fungi)...............Sub-phylum *Eumycotina* 2

2 Hyphal filaments usually coenoctytic, rarely septate, sex cells when present forming oospores or zygospores, aquatic species propagating asexually by zoospores, terrestrial species by zoospores, sporangiospores conidia or conidia-like sporangia ."Phycomycetes"... 3

The phycomycetes are generally considered to include the most primitive of the true fungi. As a whole, they encompass a wide diversity of forms with some showing relationships to the flagellates, while others closely resemble colorless algae, and still others are true molds. The vegetative body (thallus) may be non-specialized and entirely converted into a reproductive organ (holocarpic), or it may bear tapering rhizoids, or be mycelial and very extensive. The outstanding characteristics of the thallus is a tendency to be nonseptate and, in most groups, multinucliate; cross walls are laid down in vigorously growing material only to delimit the reporductive organs. The spore unit of nonsexual reproduction is borne in a sporangium, and, in aquatic and semiaquatic orders, is provided with a single posterior or anterior flagellum or two laterally attached ones. Sexual activity in the phycomycetes characteristically results in the formation of resting spores.

2' (1') Hyphal filaments when present septate, without zoospores, with or without sporangia, usually with conida; sexual reproduction absent or culminating in the formation of asci or basidia.. 8

3 (2) Flagellated cells characteristically produced..4

3' Flagellated cells lacking or rarely produced..7

4 (3) Motile cells uniflagellate...5

4' Motile cells biflagellate..6

5 (4) Zoospores posteriorly uniflagellate, formed inside the sporangium...class..*Chytridiomycetes*

The Chytridiomycetes produce asexual zoospores with a single posterior whiplash flagellum. The thallus is highly variable; the most primitive forms are unicellular and holocarpic and in their early stages of development are plasmodial (lack cell walls), more advanced forms develop rhizoids and with further evolutionary progress develop mycelium. The principle chemical component of the cell wall is chitin, but cellulose is also present. Chytrids are typically aquatic organisms but may be found in other habitats. Some species are chitinolytic and/or keratinolytic. Chytrids may be isolated from nature by baiting (e.g. hemp seeds or pine pollen) Chytrids occur both in marine and fresh water habitats and are of some economic importance due to their parasitism of algae and animals. The genus *Dermocystidium* may be provisionally grouped with the chytrids. Species of this genus cause serious epidemics of oysters and marine and fresh water fish.

5' Zoospores anteriorly uniflagellate, formed inside or outside the sporangium............class ....Hyphochytridiomycetes

These fungi are aquatic (fresh water or marine) chytrid-like fungi whose motile cells possess a single anterior flagellum of the tinsel type (feather-like). They are parasitic on algae and fungi or may be saprobic. Cell walls contain chitin with some species also demonstrating cellulose content. Little information is available on the biology of this class and at present it is limited to less than 20 species.

6 (4') Flagella nearly equal, one whiplash the other tinsel..................class.......Oomycetes

A number of representatives of the Oomycetes have been shown to have cellulosic cell walls. The mycelium is coenocytic, branched and well developed in most cases. The sexual process results in the formation of a resting spore of the oogamous type, i.e., a type of fertilization in which two heterogametangia come in contact and fuse their contents through a pore or tube. The thalli in this class range from unicellular to profusely branched filamentous types. Most forms are eucarpic; zoospores are produced throughout the class except in the more highly advanced species. Certain species are of economic importance due to their destruction of food crops (potatoes and grapes) while others cause serious diseases of fish (e.g. *Saprolegina parasitica*). Members of the family Saprolegniaceae are the common "water molds" and are among the most ubiquitous fungi in nature. The order Lagenidiales includes only a few species which are parasitic on algae, small animals, and other aquatic life. The somatic structures of this taxon are holocarpic and endobiotic. The sewage fungi are classified in the order Leptomitales. Fungi of this order are characterized by the formation of refractile constrictions: "cellulin plugs" occur throughout the thalli or, at least, at the bases of hyphae or to cut off reproductive structures. Leptomitus lacteus may produce rather extensive fouling flocs or slimes in organically enriched waters.

6' Flagella of unequal size, both whiplash .... class... Plasmodiophoromycetes

Members of this class are obligate endoparasites of vascular plants, algae, and fungi. The thallus consists of a plasmodium which develops within the host cells. Nuclear division at some stages of the life cycle is of a type found in no other fungi but known to occur in protozoa. Zoosporangia which arise directly from the plasmodium bear zoospores with two unequal anterior falgella. The cell walls of these fungi apparently lack cellulose.

7 (3') Mainly saprobic, sex cell when present a zygospore.................. class ... Zygomycetes

This class has well developed mycelium with septa developed in portions of the older hyphae; actively growing hyphae are normally non-septate. The asexual spores are non-motile sporangiospores (aplanospores). Such spores lack flagella and are usually aerialy disseminated. Sexual reproduction is initiated by the fusion of two gametangia with resultant formation of a thick-walled, resting spore, the zygospore. In the more advanced species, the sporangia or the sporangiospores are conidia-like. Many of the Zygomycetes are of economic importance due to their ability to synthesize commercially valuable organic acids and alcohols, to transform steroids such as cortisone, and to parasitize and destroy food crops. A few species are capable of causing disease in man and animals (zygomycosis).

7' Obligate commensals of arthropods, zygospores usually lacking ...... class.... Trichomycetes

The Trichomycetes are an ill-studied group of fungi which appear to be obligate commensals of arthropods. The trichomycetes are associated with a wide variety of insecta, diplopods, and crustacea of terrestrial and aquatic (fresh and marine) habitats. None of the members of this class have been cultured in vitro for continued periods of times with any success. Asexual reproduction is by means of sporangiospores. Zygospores have been observed in species of several orders.

8 (2') Sexual spores borne in asci.................................... class....... Ascomycetes

In the Ascomycetes the products of meiosis, the ascospores, are borne in sac like structures termed asci. The ascus usually contains eight ascospores, but the number produced may vary with the species or strain. Most species produce extensive septate mycelium. This large class is divided into two subclasses on the presence or absence of an ascocarp. The Hemiascomycetidae lack an ascocarp and do not produce ascogenous hyphae; this subclass includes the true yeasts. The Euascomycetidae usually are divided into three series (Plectomycetes, Pyrenomycetes, and Discomycetes) on the basis of ascocarp structure.

8' Sexual spores borne on basidia.................................. class..... Basidiomycetes

The Basidiomycetes generally are considered the most highly evolved of the fungi. Karyogamy and meiosis occur in the basidium which bears sexual exogenous spores, basidiospores. The mushrooms, toadstools, rusts, and smuts are included in this class.

8'' Sexual stage lacking........................ Form class.(Fungi Imperfecti)..Deuteromycetes

The Deuteromycetes is a form class for those fungi (with morphological affinities to the Ascomycetes or Basidiomycetes) which have not demonstrated a sexual stage. The generally employed classification scheme for these fungi is based on the morphology and color of the asexual reproductive stages. This scheme is briefly outlined below. Newer concepts of the classification based on conidium development after the classical work of S. J. Hughes (1953) may eventually replace the gross morphology system (see Barron 1968).

KEY TO THE FORM-ORDERS OF THE *FUNGI IMPERFECTI*

1	Reproduction by means of conidia, oidia, or by budding	2
1'	No reproductive structures present	*Mycelia Sterilia*
2 (1)	Reproduction by means of conidia borne in pycnidia	*Sphaeropsidales*
2'	Conidia, when formed, not in cycnidia	3
3 (2')	Conidia borne in acervuli	*Melanconiales*
3'	Conidia borne otherwise, or reproduction by oidia or by budding	Moniliales

KEY TO THE FORM-FAMILIES OF THE *MONILIALES*

1	Reproduction mainly by unicellular budding, yeast-like; mycelial phase, if present, secondary, arthrospores occasionally produced, manifest melanin pigmentation lacking	2
1'	Thallus mainly filamentous; dark melanin pigments sometimes produced	3
2 (1)	Ballistospores produced	Sporobolomycetaceae
2'	No ballistospores	Cryptococcaceae
3	Conidiophores, if present, not united into sporodochia or synnemata	4
3'	Sporodochia present	*Tuberculariaceae*
3''	Synnemata present	*Stilbellaceae*
4 (3)	Conidia and conidiophores or oidia hyaline or brightly colored	*Moniliaceae*
4'	Conidia and/or conidiophores, containing dark melanin pigment	*Dematiaceae*

SELECTED REFERENCES

Ahearn, D.G., Roth, F.J. Jr., Meyers, S.P. Ecology and Characterization of Yeasts from Aquatic Regions of South Florida. Marine Biology 1:291-308. 1968

Alexopoulos, J.C. Introductory Mycology. 2nd ed. John Wileyand Sons, New York, 613 pp. 1962

Barron, G.L. The Genera of Hyphomycetes from Soil. Williams and Wilkins Co., Baltimore. 364 pp. 1968

Cooke, W.B. Population Effects on the Fungus Population of a Stream. Ecology 42:1-18. 1961

__________. A Laboratory Guide to Fungi in Polluted Waters, Sewage, and Sewage Treatment Systems. U. S. Dept. of Health, Education and Welfare, Cincinnati, 132 pp. 1963

__________. Fungi in Sludge Digesters. Purdue Univ. Proc. 20th Industrial Waste Conference, pp 6-17. 1965a

__________. The Enumeration of Yeast Populations in a Sewage Treatment Plant. Mycologia 57:696-703. 1965b

__________. Fungal Populations in Relation to Pollution of the Bear River, Idaho-Utah. Utah Acad. Proc. 44(1):298-315. 1967

__________ and Matsuura, George S. A Study of Yeast Populations in a Waste Stabilization Pond System. Protoplasma 57:163-187. 1963

__________, Phaff, H.J., Miller, M.W., Shifrine, M., and Knapp, E. Yeasts in Polluted Water and Sewage. Mycologia 52:210-230. 1960

Emerson, Ralph and Weston, W.H. Aqualinderella fermentans Gen. et Sp. Nov., A Phycomycete Adapted to Stagnant waters. I. Morphology and Occurrence in Nature. Amer. J. Botany 54:702-719. 1967

Hughes, S.J. Conidiophores, Conidia and Classification. Can. J. Bot. 31:577-659. 1953

Johnson, T.W., Jr. Saprobic Marine Fungi. pp. 95-104. In Ainsworth, G.C. and Sussman, A.S. The Fungi, III. Academic Press, New York. 1968

__________ and Sparrow, F.K., Jr. Fungi in Oceans and Estuaries. Weinheim, Germany. 668 pp. 1961

Meyers, S.P. Observations on the Physiological Ecology of Marine Fungi. Bull. Misaki Mr. Biol. Inst. 12:207-225. 1968

Prigsheim, E.G. Iron Bacteria. Biol. Revs. Cambridge Phil. Soc. 24:200-245. 1949

Sparrow, F.K., Jr. Aquatic Phycomycetes. 2nd ed. Univ. Mich. Press, AnnArbor. 1187 pp. 1960.

__________. Ecology of Freshwater Fungi. pp. 41-93. In Ainsworth, G.C. and Sussman, A.S. The Fungi, III. Acad. Press, New York. 1968

Stokes, J.L. Studies on the Filamentous Sheathed Iron Bacterium Sphaerotilus natans. J. Bacteriol. 67:278-291. 1954

van Uden, N. and Fell, J.W. Marine Yeasts. pp. 167-201. In Droop, M.R. and Wood, E.J.F. Advances in Microbiology of the Sea, I. Academic Press, New York. 1968

Yerkes, W.D. Observations on an Occurrence of Leptomitus lacteus in Wisconsin. Mycologia 58:976-978. 1966

Cooke, William B. and Ludzack, F.J. Predacious Fungus Behavior in Activated Sludge Systems. Jour. Water Poll. Cont. Fed. 30(12):1490-1495. 1958.

PROTOZOA, NEMATODES, AND ROTIFERS

I GENERAL CONSIDERATIONS

A Microbial quality constitutes only one aspect of water sanitation; microchemicals and radionuclides are attracting increasing amount of attention lately.

B Microbes considered here include bacteria, protozoa, and microscopic metazoa; algae and fungi excluded.

C Of the free-living forms, some are members of the flora and fauna of surface waters; others washed into the water from air and soil; still others of wastewater origin; nematodes most commonly from sewage effluent.

D Hard to separate "native" from "foreign" free-living microbes, due to close association of water with soil and other environments; generally speaking, bacteria adapted to water are those that can grow on very low concentrations of nutrient and zoomicrobes adapted to water are those that feed on algae, and nematodes, especially bacteria eaters, are uncommon in water but in large numbers in sewage effluent.

E More species and lower densities of microbes in clean water and fewer species and higher densities in polluted water.

F Pollution-tolerance or nontolerance of microbes closely related to the DO level required in respiration.

G From pollution viewpoint, the following groups of microbes are of importance: Bacteria, Protozoa, Nematoda, and Rotifera.

II BACTERIA

A No ideal method for studying distribution and ecology of bacteria in freshwater.

B According to Collins,(9) *Pseudomonas*, *Achrombacter*, *Alcaligenes*, *Chromobacterium*, *Flavobacterium*, and *Micrococcus* are the most widely distributed and may be considered as indigenous to natural waters. Sulfur and iron bacteria are more common in the bottom mud.

C Actinomycetes, *Bacillus* sp. *Aerogenes* sp., and nitrogen-fixation bacteria are primarily soil dwellers and may be washed into the water by runoffs.

E Nematodes are usually of aerobic sewage treatment origin.

D *E. coli*, streptococci, and *Cl. perfringens* are true indicators of fecal pollution.

III PROTOZOA

A Classification

1 Single-cell animals in the most primitive phylum (Protozoa) in the animal kingdom.

2 A separate kingdom, Protista, to include protozoa, algae, fungi, and bacteria proposed in the 2nd edition of Ward-Whipple's *Fresh-Water Biology*.(10)

3 Four subphyla or classes:

a Mastigophora (flagellates)-Subclass phytomastigina dealt with under algae; only subclass Zoomastigina included here; 4 orders:

1) Rhizomastigina - with flagellum or flagella and pseudopodia

2) Protomonadina - with 1 to 2 flagella; mostly free-living many parasitic

3) Polymastigina - with 3 to 8 flagella; mostly parasitic in elementary tract of animals and man

4) Hypermastigina - all inhabitants of alimentary tract of insects.

b Ciliophora or Infusoria (ciliates) - no pigmented members; 2 classes:

1) Ciliata - cilia present during the whole trophic life; containing majority of the ciliates

2) Suctoria - cilia present while young and tentacles during trophic life.

c Sarcodina (amoebae) - Pseudopodia (false feet) for locomotion and food-capturing; 2 subclasses:

1) Rhizopoda - Pseudopodia without axial filaments; 5 orders:

a) Proteomyxa - with radiating pseudopodia; without test or shell

b) Mycetozoa - forming plasmodium; resembling fungi in sporangium formation

c) Amoebina - true amoeba - forming lobopodia

d) Testacea - amoeba with single test or shell of chitinous material

e) Foraminifera - amoeba with 1 or more shells of calcareous nature; practically all marine forms

d Sporozoa - no organ of locomotion; amoeboid in asexual phase; all parasitic

B General Morphology

1 Zoomastigina:

Relatively small size (5 to 40 μ); with the exception of Rhizomastigina, the body has a definite shape (oval, leaf-like, pear-like, etc.); common members with 1 or 2 flagella and some with 3, 4, or more; few forming colonies; cytostome present in many for feeding.

2 Ciliophora:

Most highly developed protozoa; with few exceptions, a macro and a micro-nucleus; adoral zone of membranellae, mouth, and groove usually present in swimming and crawling forms, some with conspicuous ciliation of a disc-like anterior region and little or no body cilia (stalked and shelled forms); Suctoria nonmotile (attached) and without cytostome cysts formed in most.

3 Sarcodina:

Cytoplasmic membrane but no cell wall; endoplasm and ectoplasm distinct or indistinct; nucleus with small or large nucleolus; some with test or shell; moving by protruding pseudopodia; few capable of flagella transformation; freshwater actinopods usually sperical with many radiating axopodia; some Testacea containing symbiotic algae and mistaken for pigmented amoebae; cysts with single or double wall and 1 or 2 nuclei.

4 Sporozoa: to be mentioned later.

C General Physiology

1 Zoomastigina:

Free-living forms normally holozic; food supply mostly bacteria in growth film on surfaces or clumps relatively aerobic, therefore the first protozoa to disappear in anaerobic conditions and re-appearing at recovery; reproduction by simple fission or occasionally by budding.

2 Ciliophora:

Holozoic; true ciliates concentrating food particles by ciliary movement around the mouth part; suctoria sucking through tentacles; bacteria and small algae and protozoa constitute main food under natural conditions; some shown in laboratory to thrive on dead organic matter and serum protein; not as aerobic as flagellates - some surviving under highly anaerobic conditions, such as Metopus; reproduction by simple fission, conjugation or encystment.

3 Sarcodina:

Holozoic; feeding through engulfing by pseudopodia; food essentially same as for ciliates; DO requirement somewhat similar to ciliates - the small amoebae and Testacea frequently present in large numbers in sewage effluent and polluted water; reproduction by simple fission and encystation.

IV NEMATODES

A Classification

1 All in the phylum Nemata (nonsegmented round worms); subdivided by some authors into two classes:

Secernentea - 3 orders:
(phasmids)

Tylenchida, Rhabditida, Strongylida, and Teratocephalida; with papillae on male tail, caudal glands absent.

Adenophora - 6 orders:
(aphasmids)

Araeolaimida, Dorylaimida, Chromdorida, Monhysterida, Enoplida, and Trichosyringida no papillae on male caudal glands absent.

2 Orders encountered in water and sewage treatment - Free-living forms inhabiting sewage treatment plants are usually bacteria-feeders and those feeding on other nematodes; those inhabiting clean waters feeding on plant matters; they fall into the following orders:

3 Tylenchida - Stylet in mouth; mostly plant parasites; some feed on nematodes, such as Aphelenchoides.

4 Rhabditida - No stylet in mouth or caudal glands in tail; mostly bacteria-feeders; common genera: Rhabiditis, Diplogaster, Diplogasteroides, Monochoides, Pelodera, Panagrellus, and Turbatrix.

5 Dorylaimida - Relatively large nematodes; stylet in mouth; feeding on other nematodes, algae and probably zoomicrobes; Dorylaimus common genus.

6 Chromadorida - Many marine forms; some freshwater dwellers feeding on algae; characterized by strong ornamentation of knobs, bristles or punctations in cuticle.

7 Monhysterida - Freshwater dwellers; esophago-intestinal valve spherical to elongated; ovaries single or paired, usually straight; common genus in water - Monhystera.

8 Enoplida - Head usually with a number of setae; Cobb reported one genus, Mononchulus, in sand filters in Washington, D.C.

B General Morphology

Round, slender, nonsegmented (transverse markings in cuticle of some) worms; some small (about $\frac{1}{2}$ mm long, as Tricephalobus), many 1 to 2 mm long (Rhabditis, Diplogaster, and Diplogasteriodes for instance), and some large (2 to 7 mm, such as Dorylaimus); sex separated but few parthenogenetic; complete alimentary canal; with elaborate mouth parts with or without stylet; complete reproductive system in each sex; no circulatory or respiratory system; complex nervous system with conspicuous nerve ring across oesophagus.

C General Physiology

1 Feeding - Most sewage treatment plant dwellers feeding on bacteria; others preying on protozoa, nematodes, rotifers,

etc., clean-water species apparently vegetarians; those with stylet in mouth use the latter to pierce the body of animal or plant and suck contents; metabolic waste mostly liquid containing ammonium carbonate or bicarbonate; enteric pathogens swallowed randomly with suspending fluid, hence remote possibility of sewage effluent-borne nematodes being pathogen-carriers.

2 Oxygen requirement - DO apparently diffused through cuticle into body; DO requirement somewhat similar to protozoa; Rhabditis tolerating reduced DO better than other Rhabditida members; all disappear under sepsis in liquid; some thrive in drying sludge.

3 Reproduction - Normal life cycle requires mating, egg with embryo formation, hatching of eggs inside or outside femals, 4 larval stages, and adult; few reproduce in the absence of males.

V ROTIFERS

A Classification:

1 Classified either as a class of the phylum Aschelminthes (various forms of worms) or as a separate phylum (Rotifera); commonly called wheel animalcules, on account of apparent circular movement of cilia around head (corona); corona contracted when crawling or swimming and expanded when attached to catch food.

2 Of the 3 classes, 2 (Seisonidea and Bdelloidea) grouped by some authors under Digononta (2 ovaries) and the other being Monogononta (1 ovary); Seisonidea containing mostly marine forms.

3 Class Digononta containing 1 order (Bdelloida) with 4 families, Philodinedae being the most important.

4 Class Monogononta comprising 3 orders: Notommatida (mouth not near center of corona) with 14 families, Floscularida Melicertida (corona with two wreaths of cilia and furrow between them) with 3 families; most import genera included in the order Notommatida: Brachionus, Keratella, Monostyla, Trichocerca, Asplanchna, Polyarthra, Synchaeta, Microcodon; common genera under the order Flosculariaceae: Floscularia, and Atrochus. Common genera under order Melicertida: Limnias and Conochilus.

5 Unfortunately orders and families of rotifers partly based on character of corona and trophi (chewing organ), which are difficult to study, esp. the latter; the foot and cuticle much easier to study.

B General Morphology and Physiology

1 Body weakly differentiated into head, neck, trunk, and foot, separated by folds; in some, these regions are merely gradual changes in diameter of body and without a separate neck; segmentation external only.

2 Head with corona, dosal antenna, and ventral mouth; mastax, a chewing organ, located in head and neck, connected to mouth anteriorly by a ciliated gullet and posteriorly to a large stomach occupying much of the trunk.

3 Common rotifers reproducing parthenogenetically by diploid eggs; eggs laid in water, cemented to plants, or carried on female until hatching.

4 Foot, a prolongation of body, usually with 2 toes; some with one toe; some with one toe and an extra toe-like structure (dorsal spur).

5 Some, like Philodina, concentrating bacteria and other microbes and minute particulate organic matter by ciliary movement on corona larger microbes chewed by mastax; some such as Monostyla feeding on clumped matter, such as bacterial growth, fungal masses, etc. at bottom; virus generally not ingested - apparently undetected by cilia.

6 DO requirement somewhat similar to protozoa; some disappearing under reduced DO, others, like Philodina, surviving at as little as 2 ppm DO.

VI SANITARY SIGNIFICANCE

A Pollution tolerant and pollution non-tolerant species - hard to differentiate - requiring specialist training in protozoa, nematodes, and rotifers.

B Significant quantitative difference in clean and polluted waters - clean waters containing large variety of genera and species but quite low in densities.

C Aerobic sewage treatment processes (trickling filters and activated sludge processes, even primary settling) ideal breeding grounds for those that feed on bacteria, fungi, and minute protozoa and present in very large numbers; effluents from such processes carrying large numbers of these zoomicrobes; natural waters receiving such effluents showing significant increase in all 3 categories.

D Possible Pathogen and Pathogen Carriers

1 *Naegleria* causing swimming associated meningto encephalitis and *Acanthameoba* causing nonswimming associated cases.

2 Amoebae and nematodes grown on pathogenic enteric bacteria in lab; none alive in amoebic cysts; very few alive in nematodes after 2 days after ingestion; virus demonstrated in nematodes only when very high virus concentrations present; some freeliving amoebae parasitizing humans.

3 Swimming ciliates and some rotifers (concentrating food by corona) ingesting large numbers of pathogenic enertic bacteria, but digestion rapid; no evidence of concentrating virus; crawling ciliates and flagellates feeding on clumped organisms.

4 Nematodes concentrated from sewage effluent in Cincinnati area showing live *E. coli* and streptococci, but no human enertic pathogens.

VII EXAMINATION OF WATER FOR MICROBES

A Bacteria - not dealt here.

B Protozoa and rotifers - should be included in examination for planktonic microbes.

C Nematodes

D Laboratory Apparatus(3)

1 *Sample Bottles* - One-gallon glass or plastic bottles with metal or plastic screw caps, thoroughly washed and rinsed three times with distilled water.

2 *Capillary Pipettes and Rubber Bulbs* - Long (9 in.) Pasteur capillary pipettes and rubber bulbs of 2 ml capacity.

3 *Filtration Unit* - Any filter holder assembly used in bacteriological examination.(1) The funnel should be at least 650 ml and the filter flask at least 2 liter capacity.

4 *Filter Membranes* - Millepore SS (SS 047 MM) type membranes or equivalent.

5 *Microscope* - Binocular microscope with 10X eyepiece, 4X, 10X, and 43X objectives, and mechanical stage.

E Collection of Water Samples

Samples are collected in the same manner(1) as those for bacteriological examination, except that a dechlorinating agent is not needed. One-half to one gallon samples are collected from raw water and one-gallon samples from tap water. Refrigeration is not essential and samples may be transported without it unless examination is to be delayed for more than five days.

F Concentration of Samples

1 One gallon of tap water can usually be filtered through a single 8-u membrane within 15 minutes unless the water has high turbidity. At least one gallon of sample should be used in a single examination. Immediately after the last of the water is disappearing from the membrane, the suction line is disconnected and the membrane placed on the wall of a clean 50 to 100 ml beaker and flushed repeatedly with about 2-5 ml of sterile distilled water

with the aid of a capillary pipette and a rubber bulb. The concentrate is then pipetted into a clean Sedgewick-Rafter Counting Cell and is ready for examination.

2 In concentration of raw water samples having visible turbidity, two to four 8-micron membranes may be required per sample, with filtration through each membrane being limited to not more than 30 minutes. Samples ranging from 500 ml to 2 liters may be filtered with one membrane, depending on degree of turbidity. After filtration the membranes are placed on the walls of separated beakers and washed as above. To prevent the particulates from obscuring the nematodes, the washing from each filter is examined in a separate counting chamber.

G Direct Microscopic Examination

Each counting chamber containing the filter concentrate is first examined under a 4X objective. Unless the concentrate contains more than 100 worms, the whole cell area is surveyed for nematodes, with respect to number, developmental stage, and motility. When an object having an outline resembling that of a nematode is observed, it is re-examined under a 10X objective for anatomical structures, unless the object exhibits typical nematode movement, which is sufficient for identifying the object as a nematode. When the concentrate contains more than 100 worms, the worm density can be estimated by counting the number of worms in representative microscopic fields and multiplying the average number of worms per field by the number of fields in the cell area. The nematode density may be expressed as number of worms per gallon with or without differentiation as to adult or larval stages or as to viability.

H General Identification of Nematodes

1 While actively motile nematodes can be readily recognized by any person who has some general concept of microscopic animals, the nonmotile or sluggishly motile nematodes may be confused with root fibers, plant filaments of various types, elongated ciliates such as Homalozoon vermiculare, or segments of appendages of small crustacea. To facilitate a general identification of nematodes, the gross morphology of three of the free-living nematodes that are frequently found in water supplies is shown in the attached drawing. The drawing provides not only the general anatomy for recognition of nematodes but also most of the essential structures for guidance to those who want to use the "Key to Genera" in chapter No. 15 on Nemata by B. G. Chitwood and M. W. Allen in the book, Fresh Water Biology.(10)

2 Under normal conditions, practically all nematodes seen in samples of finished water are in various larval stages and will range from 100 to 500 microns in length and 10 to 40 microns in width. Except in the fourth (last) stage, the larvae have no sexual organs but show other structural characteristics.

3 If identification of genera is desired, the filter washings are centrifuged at 500 rpm for a few minutes. The supernate is discarded, except a few drops, and the sediment is resuspended in the remaining water. A drop of the final suspension is examined under both 10X and 43X objectives for anatomical characteristics without staining, and for supplementary study of structures the rest is fixed in 5% formalin or other fixation fluid and stained according to instructions given in Chitwood and Allen's Chapter on Nemata,(7) Goodey's Soil and Freshwater Nematodes(11) or other books on nematology.

VIII USE OF ZOOMICROBES AS POLLUTION INDEX

A Idea not new, protozoa suggested long ago; many considered impractical because of the need of identifying pollution-intolerant and pollution-tolerant species - protozoologist required. Method also time consuming.

B Can use them on a quantitative basis - nematodes, and nonpigmented protozoa present in small numbers in clean water. Numbers greatly increased when polluted with effluent from aerobic treatment plant or recovering from sewage pollution; no significant error introduced when clean-water members included in the enumeration if a suitable method of computing the pollution index developed.

C Most practical method involves the equation: $\frac{A + B + 1000\,C}{A} = Z.P.I.$,

where

A = number of pigmented protozoa,

B = non pigmented protozoa, and

C = nematodes in a unit volume of sample, and Z.P.I. = zoological pollution index.

For relatively clean water, the value of Z.P.I. close to 1; the larger the value above 1, the greater the pollution by aerobic effluent (see attached report on zoomicrobial indicator of water pollution).

IX CONTROL

A Chlorination of effluent

B Prolongation of detention time of effluent

C Elimination of slow sand filters in nematode control.

LIST OF COMMON ZOOLOGICAL ORGANISMS FOUND IN SEWAGE TREATMENT PROCESS - TRICKLING FILTERS

PROTOZOA

Sarcodina - Amoebae

Amoeba proteus; *A radiosa*

Hartmannella

Arcella Vulgaris

Noegleria gruberi

Actinophrys ___

FLAGELLATA

Bodo caudatus

Pleuromonas jaculans

Oikomonas termo

Cercomonas longicauda

Peranema trichophorium

Swimming type

Ciliophora:

Colpidium colpoda

Colpoda cuculus

Glaucoma pyriformis

Paramecium candatum; *P bursaria*

Stalked type

Opercularia sp. (short stalk dichotomous)

Vorticella sp. (stalk single and contractile)

Epistylis plicatilis (like opercularia, more colonial, stalk not contractile)

Carchesium sp. (like vorticella but colonial, individual zooids contractile)

Zoothamnium sp. (entire colony contracts)

Crawling type

Euplotes patella

Stylonychia mylitus

Urostyla sp.

Oxytricha sp.

NEMATODA

Diplogaster sp.

Monochoides sp.

Diplogasteroides sp.

Rhabditis sp.

Pelodera sp.

Aphelenchoides sp.

Dorylamus sp.

Chlindrocorpus sp.

Cephalobus sp.

Rhabditolaimus sp.

Monhystera sp.

Trilobus sp.

ROTATORIA

Diglena

Monostyla

Polyarthra

Philodina

Keratella

Brachionus

OLIGOCHAETA (bristle worms)

Aelosoma hemprichi

Aulophorus limosa

Tubifex tubifex

Lumbricillus lineatus

INSECT LARVAE

Chironomus

Psychoda sp. (trickling filter fly)

ARTHROPODA

Lessertia sp.

Porrhomma sp.

Achoratus subuiaticus (collembola)

Folsomia sp. (collembola)

Tomocerus sp. (collembola)

REFERENCES

1 American Public Health Association, American Water Works Association and Water Pollution Control Federation. *Standard Methods for the Examination of Water and Wastewater*, 13th ed. New York. 1971.

2 Chang, S. L., et al. Survey of Free-Living Nematodes and Amoebas in Municipal Supplies. J.A.W.W.A. 52: 613-618.

3 Chang, S. L. Interactions between Animal Viruses and Higher Forms of Microbes. Proc. Am. Soc. Civ. Eng. Jl. San. Eng. 96:151. 1970

4 Chang, S. L. Zoomicrobial Indicators of Water Pollution presented at the Annual Meeting of Am. Soc. Microbial, Philadelphia, April 23-28, 1972.

5 Chang, S. L. Pathogenic Free-Living Amoebae and Recreational Waters. Presented at 6th International Conference of Water Pollution Research Association, Jerusalem, Israel, June 19-24, 1972.

6 Chang, S. L. Proposed Method for Examination of Water for Free-Living Nematodes. J.A.W.W.A. 52:695-698. 1960.

7 Chang, S. L., et al. Survival and Protection Against Chlorination of Human Enteric Pathogens in Free-Living Nematodes Isolated from Water Supplies. Am. Jour. Trop. Med. and Hyg. 9:136-142. 1960.

8 Chang, S. L. Growth of Small Free-Living Amoebae in Bacterial and Bacteria-Free Cultures. Can. J. Microbial. 6:397-405. 1960.

9 Chang, S. L. and Kabler, P. W. Free-Living Nematodes in Aerobic Treatment Plant Effluents. J.W.P.C.F. 34:1256-2161. 1963.

10 Chitwood, B.G. and Chitwood, M.B. *An Introduction to Nematology*. Section I: Anatomy. 1st ed. Monumental Printing Co. Baltimore. 1950. pp 8-9.

11 Cobb, N.A. Contributions to the Science of Nematology VII. Williams and Wilkins Co. Baltimore. 1918.

12 Collins, V.G. The Distribution and Ecology of Bacteria in Freshwater, Pts. I & II, Proc. Soc. for Water Treatment and Exam. 12:40-73. 1963. (England)

13 Edmondson, W.T., et al. Ward-Whipple's *Fresh Water Biology*. 2nd ed. John Wiley & Sons, New York. 1959. pp 368-401.

14 Goodey, T. Soil and Freshwater Nematodes. (A Monograph) 1st ed. Methuen and Co. Ltd. London. 1951.

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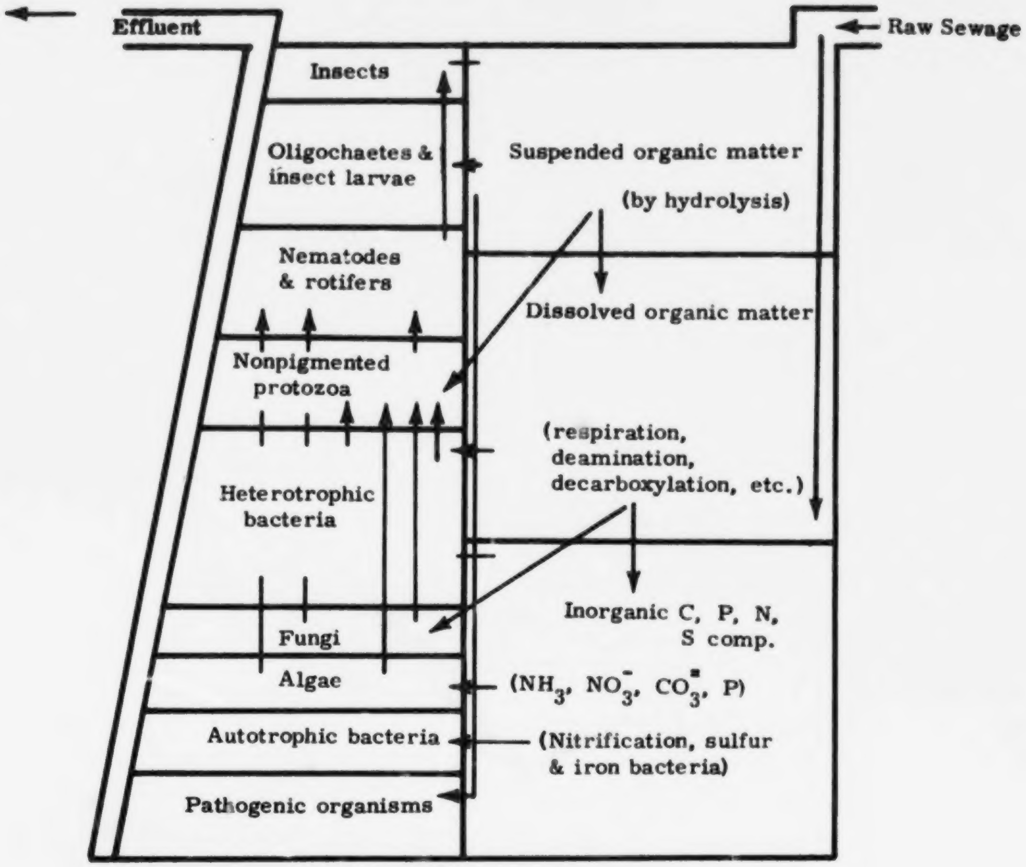
Effluent
Raw Sewage
Insects
Oligochaetes & insect larvae
Nematodes & rotifers
Nonpigmented protozoa
Heterotrophic bacteria
Fungi
Algae
Autotrophic bacteria
Pathogenic organisms
Suspended organic matter
(by hydrolysis)
Dissolved organic matter
(respiration, deamination, decarboxylation, etc.)
Inorganic C, P, N, S comp.
(NH$_3$, NO$_3^-$, CO$_3^=$, P)
(Nitrification, sulfur & iron bacteria)

Food Chain in Aerobic Sewage Treatment Processes

FREE-LIVING AMOEBAE AND NEMATODES

I FREE-LIVING AMOEBAE

A Importance of Recognizing Small, Free-Living Amoebae in Water Supplies

1 Commonly found in soil, aerobic sewage effluent and natural, fresh waters - hence, frequently encountered in examination of raw water.

2 Cysts not infrequently found in municipal supplies - not pathogen carriers.

3 Flagellate-amoebae *Naegleria* involved in 50 some cases of meningoencephalitis, about half in the U.S.; associated with swimming in small warm lakes. *Acanthamoeba rhysodes* parasitizing hyman throats and causing (3 cases) nonswimming-associated meningoencephalitis.

4 Cysts not to be confused with those of *Endamoeba histolytica* in waterborne epidemics.

B Classification of Small, Free-Living Amoebae

1 Recognized classification based on characteristics in mitosis.

2 Common species fall into the following families and genera:

Family Schizopyrenidae: Genera *Naegleria*, *Didascalus*, and *Schizopyrenus* - first two being flagellate amoebae.

Family Hartmannellidae: Genera *Hartmanella* (*Acanthamoeba*)

3 How to prepare materials for studying mitosis - Feulgen stain

C Morphological Characteristics of Small, Free-Living Amoebae

1 Morphology of Trophozoites - Ectoplasm and endoplasm usually distinct; nucleus with large nucleolus.

2 Morphology of cysts - Single or double wall with or without pores

D Cultural Characteristics of Small, Free-Living Amoebae

1 How to cultivate these amoebae - plates with bacteria; cell cultures, axenic culture.

2 Growth characteristics on plate, cell, and axenic culture

3 Complex growth requirements for most of these amoebae

E Resistance of Amoebic Cysts to Physical and Chemical Agents

II FREE-LIVING NEMATODES

A Classification of Those Commonly Found in Water Supplies

1 Phasmidia (Secerneutes): Genera *Rhabditis*, *Diplogaster*, *Diplogasteroides*, *Cheilobus*, *Panagrolaimus*

2 Aphasmidia (Adenophoro): Genera *Monhystera*, *Aphelenchus*, *Turbatrix* (vinegar eel), *Dorylaimus*, and *Rhabdolaimus*

B Morphological Features

1 Phasmids: papilla on tail of males, mouth adapted to feed on bacteria, few exceptions.

2 Aphasmids: no papilla on male tail; glandular cells in male.

C Life Cycle

  1 Methods of mating

  2 Stages of development

  3 Parthenogenesis

D Cultivation

  1 Bacteria-fed cultures

  2 Axenic cultures

E Occurrence in Water Supplies

  1 Relationship between their appearance in finished water and that in raw water.

  2 Frequency of occurrence in different types of raw water and sources.

  3 Survival of human enteric pathogenic bacteria and viruses in nematodes.

  4 Protection of human enteric pathogenic bacteria and viruses in nematode-carriers.

F Control

  1 Chlorination of sewage effluent

  2 Flocculation and sedimentation of water

  3 Chlorination of water

  4 Other methods of destruction

REFERENCES

Amoebae

1 Singh, B.N., "Nuclear Division in Nine Species of Small, Free-Living Amoebae and its Bearing on the Classification of the Order Amoebida", Philos. Trans. Royal Soc. London, Series B, 236:405-461, 1952.

2 Chang, S.L., et al. "Survey of Free-Living Nematodes and Amoebas in Municipal Supplies". J.A.W.W.A. 52:613-618, 1960.

3 Chang, S.L., "Growth of Small Free-Living Amoebae in Various Bacterial and in Bacteria-Free Cultures". Can. Jour. Microbiol. 6:397-405, 1960.

Nematodes

1 Goodey, T., "Freshwater Nematodes", 1st. Edition, Methuen & Co., London, 1951.

2 Edmondson, W.T., Ed., Ward & Whipple's "Fresh-Water Biology" 10th Edition, page 397, 1955.

3 Chang, S.L., et al., "Occurrence of a Nematode Worm in a City Water Supply". J.A.W.W.A., 51:671-676, 1959.

4 Chang, S.L., et al., "Survival, and Protection Against Chlorination, of Human Enteric Pathogens in Free-Living Nematodes Isolated From Water Supplies". Am. Jour. Trop. Medicine & Hygiene, 9:136-142, 1960.

5 Chang, S.L., et al., "Survey of Free-Living Nematodes and Amoebas in Municipal Supplies". J.A.W.W.A., 52:613-618, 1960.

6 Chang, S.L., "Proposed Method for Examination of Water for Free-Living Nematodes". J.A.W.W.A., 52:695-698, 1960.

7 Chang, S.L., "Viruses, Amoebas, and Nematodes and Public Water Supplies". J.A.W.W.A., 53:288-296, 1961.

8 Chang, S.L., and Kabler, P.W., "Free-Living Nematodes in Sewage Effluent from Aerobic Treatment Plants". To be published.

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SUGGESTED CLASSIFICATION OF SMALL AMOEBAE

Subphylum: Sarcodina Hertwig and Lesser

Class: Rhizopoda von Siebold

Subclass: Amoebaea Butschli

Order: Amoebida Calkins and Ehrenberg

Superfamily: Amoebaceae - free-living
(Endamoebaceae - parasitic in animals)

Family: Schizopyrenidae - active *limax* form common; transcient flagellates present or absent; nucleonus-origin of polar masses; polar caps and interzonal bodies present or absent

Genus: *Schizopyrenus* - no transcient flagellates; single-walled cysts; no polar caps or interzonal bodies in mitosis

Species: *S. erythaenusa* - reddish orange pigment formed in agar cultures with gram-negative bacillary bacteria

S. russelli - no pigment produced in agar cultures

Genus: *Didascalus* - morphology and cytology similar to *Schizopyrenus* but small numbers of transcient flagellates formed at times

Species: *D. thorntoni* - only species described by Singh (1952)

Genus: *Naegleria* Alexeieff - double-walled cysts; transcient flagellates formed readily; polar caps and interzonal bodies present in mitosis

Species: *N. gruberi* (Schardinger) - only species established; Singh (1952) disclaimed the *N. soli* he described in 1951

Family: Hartmannellidae - no transcient flagellate formed; motility sluggish; no *limax* form; nucleolus disappearing, probably forming spindle in mitosis; no polar caps or masses, aster and centrosome not known

Genus: *Hartmannella* - ectoplasm clear or less granular than endoplasm, single-walled cysts; single vacuole

Species: *H. glebae* - clear ectoplasm
H. agricola - ectoplasm less granular than endoplasm

Genus: *Acanthamoeba* - filamentous processes from ecto- or endoplasm; growing axenically in fluid bacteriological media

Species: *A. rhysodes*

Genus: *Singhella* - double-walled cysts; ecto- and endoplasm indistinguishable; many vacuoles

Species: *Singhella leptocnemus*

ANIMAL PLANKTON

I INTRODUCTION

A Planktonic animals or zooplankton are found in nearly every major group of animals.

1 Truly planktonic species (euplankton) spend all or most of their active life cycle suspended in the water. Three groups are predominantly involved in fresh water; the protozoa, rotifers, and microcrustacea.

2 Transient planktonic phases such as floating eggs and cysts, and larval stages occur in many other groups.

B Many forms are strictly seasonal in occurrence.

C Certain rare forms occur in great numbers at unpredictable intervals.

D Techniques of collection, preservation, and identification strongly influence the species reported.

E In oceanographic work, the zooplankton is considered to include many relatively large animals such as siphonophores, ctenophores, hepteropods, pteropods, arrowworms, and euphausid shrimp.

F The plant-like or phytoplankton on the other hand are essentially similar in all waters, and are the nutritional foundation for the animal community.

II PHYLUM PROTOZOA

A The three typically free living classes, Mastigophora, Rhizopoda, and Ciliophora, all have planktonic representatives. As a group however, the majority of the phylum is benthic or bottom-loving. Nearly any of the benthic forms may occasionally be washed up into the overlying waters and thus be collected along with the euplankton.

B Class mastigophora, the nonpigmented zooflagellates.

These have frequently been confused with the phytomastigina or plant-like flagellates. The distinction is made here on the basis of the presence or absence of chlorophyll as suggested by Palmer and Ingram 1955.

(Note Figure: Nonpigmented, Non-Oxygen Producing Protozoan Flagellates in the outline Oxygen Relationships.)

1 Commonly encountered genera

Bodo

Peranema

2 Frequently associated with eutrophic conditions

C Class Rhizopoda - amoeboid protozoans

1 Forms commonly encountered as plankton:

Chaos

(Amoeba)

Arcella

Centropyxis

Difflugia

Heliozoa

Euglypha

2 Cysts of some types may be encountered in water plants or distribution systems; rarely in plankton of open lakes or reservoirs.

D Class Ciliophora

1 Certain "attached" forms often found floating freely with plankton:

Vorticella

Carchesium

2 Naked, unattached ciliates. Halteria one of commonest in this group. Various heavily ciliated forms (holotrichs) may occur from time to time such as Colpidium, Enchelys, etc.

3 Ciliates protected by a shell or test (testaceous) are most often recorded from preserved samples. Particularly common in the experience of the National Water Quality Sampling Network are:

Codonella fluviatile

Codonella cratera

Tintinnidium (usually with organic matter)

Tintinnopsis

III PHYLUM ROTIFERA

A Some forms such as *Anuraea cochlearis* and *Asplanchna pridonta* tend to be present at all times of the year. Others such as *Notholca striata*, *N. longispina* and *Polyarthra platyptera* are reported to be essentially winter forms.

B Species in approximate order of descending frequency currently recorded by National Water Quality Sampling Network are:

Keratella cochlearis

Polyarthra vulgaris

Synchaeta pectinata

Brachionus quadridentata

Trichocerca longiseta

Rotaria sp.

Filinia longiseta

Kellicottia longispina

Pompholyx sp.

C Benthic species almost without number may be collected with the plankton from time to time.

IV PHYLUM ARTHROPODA

A Class Crustacea

1 The Class Crustacea includes the larger common freshwater euplankton. They are also the greatest planktonic consumers of basic nutrients in the form of phytoplankton, and are themselves the greatest planktonic contribution to the food of fishes. Most of them are herbivorous. Two groups, the cladocera and the copepods are most conspicuous.

2 Cladocera (Subclass Branchiopoda, Order Cladocera) or Water Fleas

a Life History

1) During most of the year, eggs which will develop without fertilization (parthenogenetic) are deposited by the female in a dorsal brood chamber. Here they hatch into minature adults which escape and swim away.

2) As unfavorable conditions develop, males appear, and thick-walled sexual eggs are enclosed in egg cases called ephippia which can often endure freezing and drying.

3) Sexual reproduction may occur at different seasons in different species.

4) Individuals of a great range of sizes, and even ephippia, are thus encountered in the plankton, but there is no "larval" form.

b Seasonal variation - Considerable variation may occur between winter and summer forms of the same species in some cases. Similar variation also occurs between arctic and tropical situations.

c Forms commonly encountered as open water plankton include:

Bosmina longirostris and others

Daphnia galeata and others

Other less common genera are:

Diaphanosoma, *Chydorus*, *Sida*, *Acroperus*, *Ceriodaphnia*, *Bythotrephes*, and the carnivorous *Leptodora* and *Polyphemus*.

d Heavy blooms of Cladocerans may build up in eutrophic waters.

3 The copepods (order Copepoda) are the perennial microcrustacea of open waters, both fresh and marine. They are the most ubiquitous of animal plankton.

a *Cyclops* is the genus most often found by the National Water Quality Sampling Network activities. *Eucyclops*, *Paracyclops*, *Diaptomus*, *Canthocamptus*, *Epischura*, and *Limnocalanus* are other forms reported to be planktonic.

b Copepods hatch into a minute characteristic larvae called a nauplius which differs considerably from the adults. After five or six moults, the copepodid stage is reached, and after six more moults, the adult. These larval stages are often encountered and are difficult to identify.

B Class Insecta

1 Only a single species of insect can be ranked as a true plankton, this is the midge fly Chaborus (approx. 8 spp, formerly Corethra).

2 The larva of this insect has hydrostatic organs that enable it to remain permanently suspended in the water.

3 It is usually found in the depths during the daytime, but comes to the surface at night.

V OCCASIONAL PLANKTERS

A While the protozoa, rotifers, and microcrustacea make up the bulk of the plankton, there are many other groups as mentioned above that may also occur. Locally or periodically these may be of major importance. Examples are given below.

B Phylum Coelenterata

1 Polyps of the genus Hydra may become detached and float about hanging from the surface film or floating detritus.

2 The freshwater medusa Craspedacusta occasionally appears in lakes or reservoirs in great numbers.

C Phylum Platyhelminthes

1 Minute Turbellaria (relatives of the well known Planaria) are sometimes taken with the plankton in eutrophic conditions. They are readily confused with ciliate protozoa.

2 Cercaria larvae of Trematodes (flukes) parasitic on certain wild animals, frequently appear in great numbers. When trapped in the droplets of water on a swimmer's skin, they attempt to bore in. Man not being their natural host, they fail. The resultant irritation is called "swimmer's itch". Some can be identified, but many unidentifiable species may be found.

3 In many areas of the world, cercaria larvae of human parasites such as the blood fluke Schistosoma japonicum may live as plankton, and penetrate the human skin directly on contact.

D Phylum Nemathelminthes

1 Nematodes (or nemas) or roundworms approach the bacteria and the blue-green algae in ubiquity. They are found in the soil and in the water, and in the air as dust. In both marine and fresh waters and from the Arctic to the tropics.

2 Although the majority are free living, some occur as parasites of plants, animals, and man, and some of these parasites are among out most serious.

3 With this distribution, it is obvious that they will occasionally be encountered as plankton. A more complete discussion of nematodes and their public health implications in water supplies will be found elsewhere (Chang, S. L.).

E Additional crustacean groups sporadically met with in the plankton include the following:

1 Order Anostraca or fairy shrimps (formerly included with the two following orders in the Euphyllopoda) primarily planktonic in nature.

a Extremely local and sporadic, but when present, may be dominating.

b Artemia, the brine shrimp, can tolerate very high salinities.

c Very widely distributed, poorly understood.

2 Order Notostraca, the tadpole shrimps. Essentially southern and western in distribution.

3 Order Conchostraca, the clam shrimps. Widely distributed, sporadic in occurrence. Many local species.

4 Subclass Ostracoda, the seed shrimps. Up to 3 in. in length. Essentially benthic but certain species of Cypris, and Notodromas may occur in considerable numbers as plankton at certain times of the year.

5 Certain members of the large subclass Malacostraca are limnetic, and thus, planktonic to some extent.

a The scuds, (order Amphipoda) are essentially benthic but are sometimes collected in plankton samples around weed beds or near shore. Nektoplanktonic forms include Pontoporeia and some species of Gammarus.

b The mysid, or opossum shrimps are represented among the plankton by Mysis relicta, which occurs in the deeper waters, large lakes as far north as the Arctic Ocean.

F The Class Archnoidea, Order Hydracarina (or Acari) the mites. Frequent in plankton tows near shore although Unionicola crassipes has been reported to be virtually planktonic.

G The phylum Mollusca is but scantily represented in the freshwater plankton, in contrast to the marine situation. Glochidia (ciliated) larvae are occasionally collected, and snails now and then glide out on a quiet surface film and are taken in a plankton net. An exotic bivalve Corbicula has a planktotrophic veliger stage.

H Eggs and other reproductive structures of many forms including fish, insects, and rotifers may be found in plankton samples. Special reproductive structures such as the statoblasts of bryozoa and sponges, and the ephippia of cladocerans may also be included.

I Adventitious and Accidental Plankters

Many shallow water benthic organisms may become accidentally and temporarily incorporated into the plankton. Many of those in the preceding section might be listed here, in addition to such forms as certain free living nematodes, small oligochaetes, and tardigrades, Collembola and other surface film livers are also taken at times but should not be mistaken for plankton. Fragments and molt skins from a variety of arthropods are usually observed.

Pollen from terrestrial or aquatic plants is often unrecognized, or confused with one of the above. Leaf hairs from terrestrial plants are also confusing to the uninitiated, they are sometimes mistaken for fungi or other organisms (and vice versa).

In flowing waters, normally benthic (bottom living) organisms are often found drifting freely in the stream. This phenomenon may be constant or periodic. When included in plankton collections, they must be reported, but recognized for what they are.

REFERENCES

1 Edmondson, W.F., ed. Ward and Whipple's Freshwater Biology, 2nd Edition, Wiley & Sons, Inc., New York. 1959.

2 Hutchinson, G. Evelyn. A Treatise on Limnology. Vol. 2. Introduction to Lake Biology and the Limnoplankton. Wiley. 1115 pp. 1967.

3 Lackey, J.B. Quality and Quantity of Plankton in the South End of Lake Michigan in 1952. JAWWA. 36:669-74. 1944.

4 McGauhey, P.H., Eich, H.F., Jackson, H.W., and Henderson, C. A Study of the Stream Pollution Problem in the Roanoke, Virginia, Metropolitan District. Virginia Polytech. Inst., Engr. Expt. Sta.

5 Needham, J.G. and Lloyd, J.T. The Life of Inland Waters. Ithaca, New York, Comstock Publishing Co., Inc. 1937.

6 Newell, G.E. and Newell, R.C. Marine Plankton. Hutchinson Educ. Ltd. London. 221 pp. 1963.

7 Palmer, C.M. and Ingram, W.M. Suggested Classification of Algae and Protozoa in Sanitary Science. Sew. & Ind. Wastes. 27:1183-88. 1955.

8 Pennak, R.W. Freshwater Invertebrates of the United States. The Ronald Press, New York. 1953.

9 Sverdrup, H.W., Johnson, M.W., and Fleming, R.H. The Oceans, Their Physics, Chemistry and General Biology. Prentice-Hall, Inc., New York. 1942.

10 Welch, P.S. Limnology, McGraw-Hill Book Co., Inc., New York. 1935.

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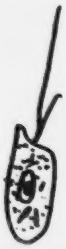
Phylum PROTOZOA

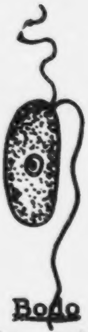
3/4

Free Living Representatives

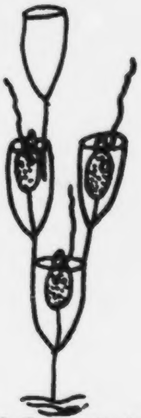
I. Flagellated Protozoa, Class Mastigophora



Anthophysis
Pollution tollerant
6 µ

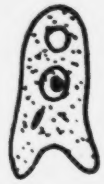


Bodo
Pollution tollerant
19 µ

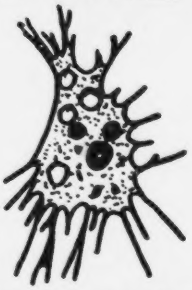


Colony of Poteriodendron
Pollution tollerant, 35 µ

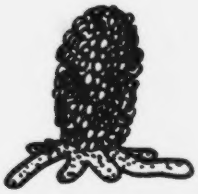
II. Ameboid Protozoa, Class Sarcodina



Dimastigamoeba
Pollution tollerant
10-50 µ

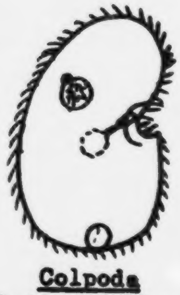


Nuclearia, reported
to be intollerant of
pollution, 45 µ.

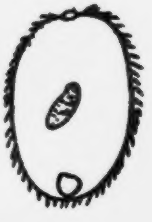


Difflugia
Pollution tollerant
60-500 µ

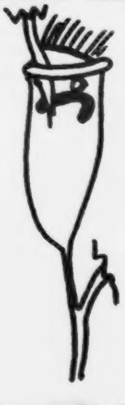
III. Ciliated Protozoa, Class Ciliophora



Colpoda
Pollution tollerant
20-120 µ



Holophrya, reported
to be intollerant of
pollution, 35 µ



Epistylis, pollution
tollerant. Colonies often
macroscopic.

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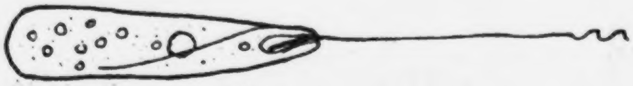
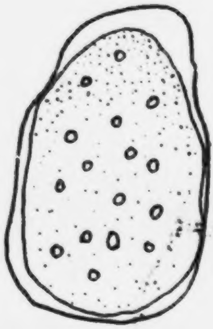
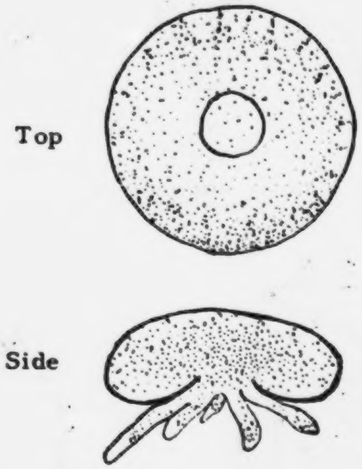
PLANKTONIC PROTOZOA



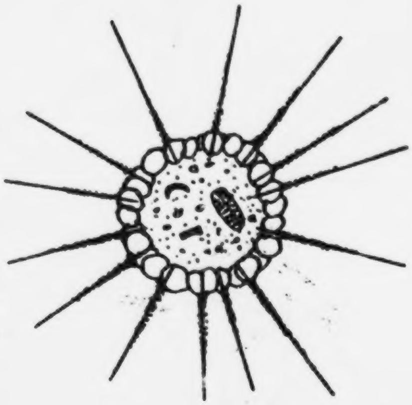
Peranema trichophorum



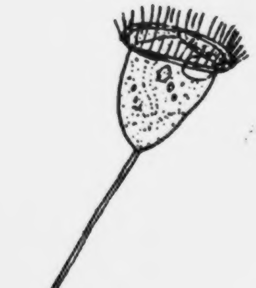
Chaos



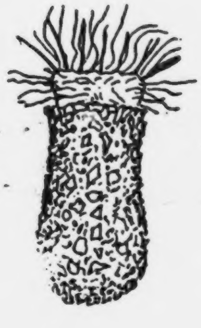
Arcella
vulgaris



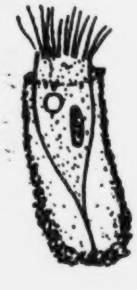
Actinosphaerium



Vorticella

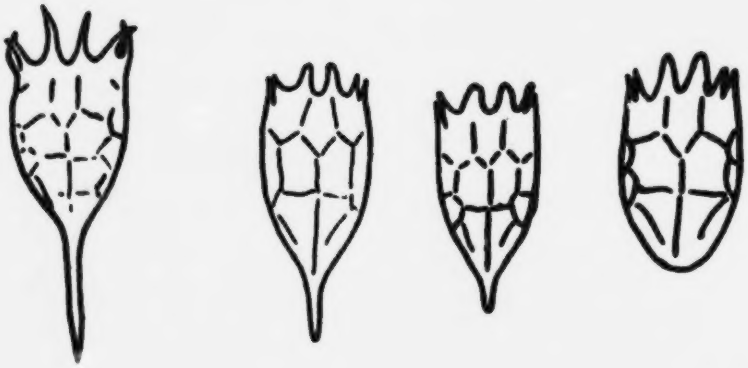


Codonella
cratera

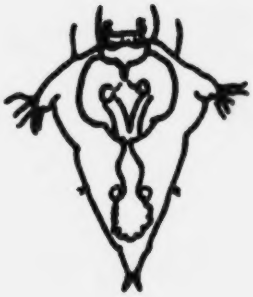


Tintinnidium
fluviatile

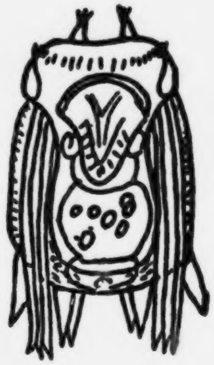
PLANKTONIC ROTIFERS



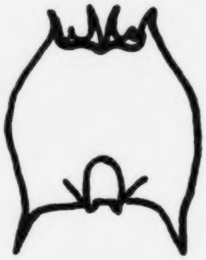
Various Forms of Keratella cochlearis



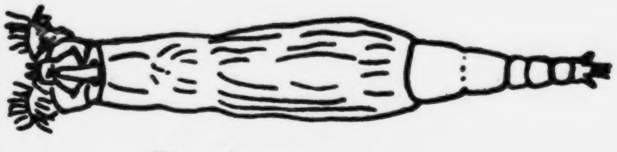
Synchaeta pectinata



Polygarthra vulgaris



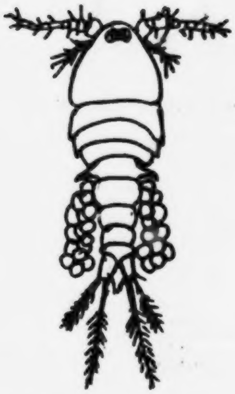
Brachionus quadridentata



Rotaria sp

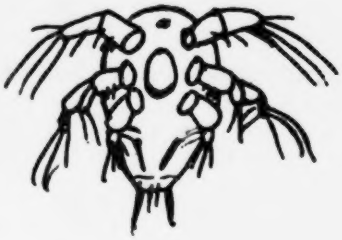
SOME PLANKTONIC CRUSTACEANS

CRUSTACEANS



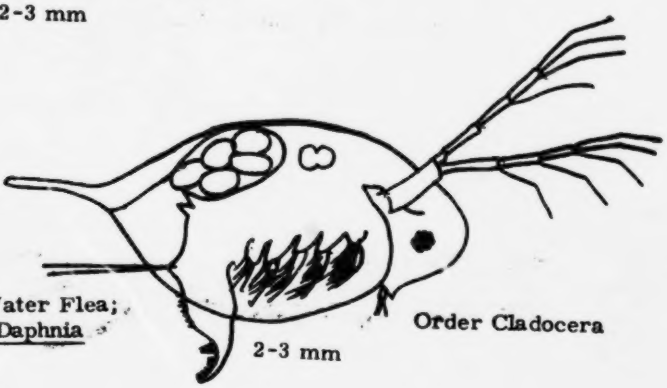
Copepod; Cyclops, Order Copepoda

2-3 mm



A Nauplius larva of a Copepod

1-5 mm

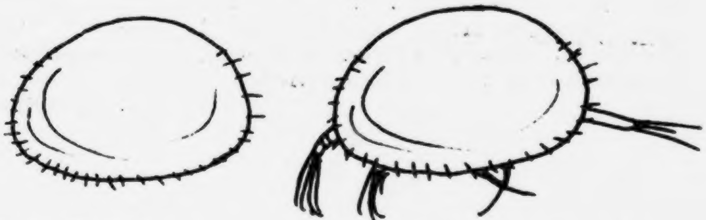


Water Flea; Daphnia

2-3 mm

Order Cladocera

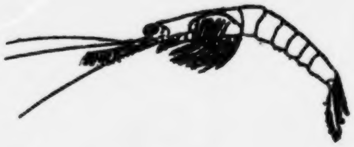
OSTRACODE



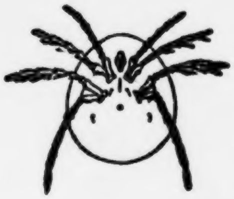
Left: Shell closed Right: Appendages extended

1-2 mm

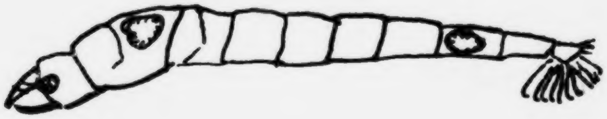
PLANKTONIC ARTHROPODA



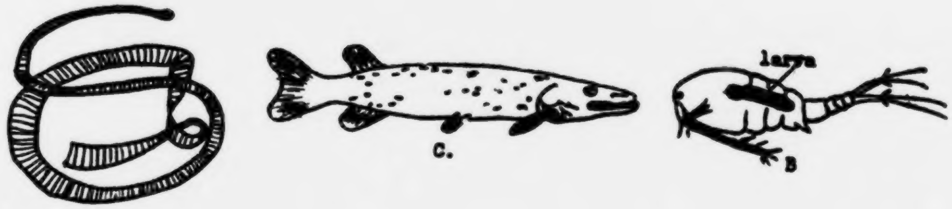
A mysid shrimp - crustacean



A water mite - arachnid



Chaoborus midge larva - Insect



Aspects in the life cycle of the human tapeworm Diphyllobothrium latum, class Cestoda. A. adult as in human intestine; B. procercoid larva in copepod; C. plerocercoid larva in flesh of pickerel (X-ray view).

H.W. Jackson

LABORATORY: IDENTIFICATION OF DIATOMS

I OBJECTIVES

A To become familiar with important structural features of diatoms.

B To learn to recognize some common forms at sight.

C To learn to identify less common forms using technical keys.

II PROCEDURE

A Transfer a drop of the water sample containing diatoms to a microscope slide. Cover with cover glass and observe under low power (10X) of microscope.

1 Do all of the diatom cells appear to have the same shape? Do some have square ends and some rounded ends? Touch the cover glass with your pencil several times as you observe through the microscope and note the relationship of the two types of ends to one another.

2 Find a place where a round-ended and a square-ended cell are close together and observe these under oil power (90X). The round-ended view is that of the top or bottom of the diatom cell and is called the "valve" view. The square ended view is that of the side of the cell and is called the "girdle" view.

3 In the valve view note the cross lines in the wall. In this diatom there are many fine lines and a smaller number of coarse lines. The former are present in all diatoms and are called "striae" or "striations." The latter are present only in some genera of diatoms and are called "septa," or in other genera, "costae." Which of the two types of lines are continuous from side to side? The space left in the center by the interrupted lines is known as a "false (pseudo) raphe."

4 What is the predominant color of the diatom? How many plastids? In diatoms, the identification is based almost entirely on the characteristics of the cell wall.

5 Make an outline drawing, at least 3 inches long, of a valve view and a girdle view of the diatom. Show the markings in the upper third of each. Label the striae, septa, and false-raphe. Make a drawing of what you image an end view or cross(transverse) section view would be like.

6 Using the key, identify your specimen, listing the alternatives selected.

B Use the key to identify other unknowns as far as possible, listing the alternatives selected in the key. Make a sketch of Navicula and Cyclotella if you identify these forms.

III IMPORTANT TERMS

Capitate - having a known-like end.

Costae - coarse transverse ribs in wall.

False raphe - (see pseudoraphe)

Frustule - the wall of the diatom.

Girdle view - the side view, in which the diatom appears to have square or blunt ends.

Nodule - a lump-like swelling in the center or ends of the valve.

Pseudoraphe - a clear space extending the length of the diatom and bordered on both sides by striae.

Punctae - the dots which comprise the striae.

Raphe - a longitudinal line (cleft) bordered on both sides of striae.

Septa - a self-like partition in the diatom, appearing often as a coarse line.

Striae - fine transverse lines especially evident in the valve view.

Valve view - the top or bottom view, in which the diatom has rounded ends, or is circular in outline.

REFERENCES

1 Boyer, C.S. The Diatomaceae of Philadelphia and Vicinity. J.B. Lippincott Co. Philadelphia. 1916. p 143.

2 Boyer, C.S. Synopsis of North America Diatomaceae, Parts I (1927) and II (1928). Proceedings of the Academy of Natural Sciences, Philadelphia.

3 Elmore, C.J. The Diatoms of Nebraska. University of Nebraska Studies, 21:22-215. 1921.

4 Hohn, M.H. A Study of the Distribution of Diatoms in Western New York State. Cornell University Agricultural Experimental Station. Memoir 308, pp 1-39. 1951.

5 Pascher, A. Bacillariophyta (Diatomeae). Heft 10 in Die Susswasser-Flora Mitteleuropas, Jena. 1930. p 466.

6 Patrick, R. A Taxonomic and Ecological Study of Some Diatoms from the Pocono Plateau and Adjucant Regions, Farlowia. 2:143-221. 1945.

7 Smith, G.M. Class Bacillariophyceae. Freshwater Algae of the United States, McGraw-Hill Book Co. New York. pp 440-510 2nd Ed. 1950.

8 Tiffany, L.H., and Britton, M.E. Class Bacillariophyceae. The Algae of Illinois, University of Chicago Press. pp 214-296. 1952.

9 Ward, H.B., and Whipple, G.C. Class I, Bacillariaceae (Diatoms). Freshwater Biology, J. Wiley & Sons. New York. pp 171-189. 1948.

10 Weber, C.I. A Guide to the Common Diatoms at Water Pollution Surveillance System Stations. USDI. FWPCA, Cincinnati, OH. 1966.

11 Whipple, G.C., Fair, G.M., and Whipple, M.C. Diatomaceae. Microscopy of Drinking Water. Chapter 21, 4th ed. J. Wiley and Sons, New York. 1948.

This outline was prepared by L.G. Williams, Aquatic Biologist, Formerly with Research and Development, Cincinnati Water Research Laboratory, FWPCA, SEC.

PREPARATION OF PERMANENT DIATOM MOUNTS

I The identification of many diatoms to genus and all diatoms to species requires that the cells be free of organic contents. This is necessary because the taxonomy of the diatoms is based on the structure of the frustule (shells) of the organisms and many features are masked by the presence of organic materials which may remain inside. It is also necessary that at least 1000× magnification (oil immersion) be used to detect the structural features used in identification. No simple procedure for the accurate routine counting of diatoms has yet been developed.

II MATERIALS NECESSARY

A Sample Concentration

1 Centrifuge (such as Universal DU)

2 100 ml centrifuge tubes

3 Membrane filter apparatus

4 Vacuum

B Slide Preparation

1 Slides, 1 × 3 inch, frosted-end

2 Cover glasses, circular #1, 18 mm, 0.13 - .16 mm thick

3 Resinous mounting medium (such as Harleco microscope mounting medium)

4 Hot plates

  a 180° F

  b 700° F

5 Disposable pipettes

6 3 × 6 × 1/4 inch steel plate

III PROCEDURE

A The volume of sample needed will vary according to the density of diatoms and silt, and only with experience can the correct sample size be determined. In most cases, 100 ml will be sufficient.

1 Spin 100 ml at 1000 G for 20 minutes.

2 Withdraw the supernatant liquid with an aspirator, being careful not to disturb the concentrate at the bottom of the centrifuge tube. (Draw off all but 2-3 ml.)

3 Transfer the concentrate to a labelled 10 ml disposable vial. Label the vial with a magic marker, diamond pencil, or "time" label.

4 If the sample has been preserved with formalin, or contains more than 1.0 gram per liter dissolved solids, it will be necessary to wash the concentrate with distilled water. In this case, transfer the entire concentrate to a 15 ml centrifuge tube. Dilute to 15 ml with distilled water, making certain that the sample is well mixed. Spin for 10 minutes at full speed in a clinical centrifuge. Withdraw the supernatant liquid, and refill with distilled water. Spin again for 10 minutes. Withdraw the supernatant liquid as before, return the concentrate to the rinsed vial in 2-3 ml of distilled water and proceed with the mounting.

5 If more than 200 ml of sample must be centrifuged to obtain sufficient material to prepare a diatom slide, concentrate the diatoms by filtering the sample through a 1.2 micron pore diameter membrane filter. Transfer the filter to a 15 ml centrifuge tube, and dissolve with 90% acetone. Centrifuge 10 minutes (full speed) and decant with an aspirator. Refill with 90% acetone,

agitate, and spin again for 10 minutes. Repeat until three fresh acetone washes have been used. Replace the acetone with 2-3 ml of distilled water and transfer to a labelled vial as described in #4.

B If the loss of minute forms in supernatant is suspected, spin 100 ml at 1000 gs in a batch centrifuge for as long as may be necessary, then proceed as below.

C Mounting

1 Heat the hot plates to the prescribed temperatures.

2 Place one cover glass on the steel plate for each sample.

3 Place the steel plate on the 180° F hot plate.

4 Transfer a drop of sample to a cover glass.

5 Allow the water to evaporate (caution: do not allow it to boil.)

6 Continue to add more sample until a thin layer of material is noticeable on the dry cover glass, or until all of the concentrate has been used. This step is especially critical, and can be learned only by trial and error.

7 Transfer the steel plate to the 700° F hot plate for 20-30 minutes. (The plate should be hot enough to incinerate paper.)

8 While the material is on the high temperature hot plate, label the microscope slides (use a #2 pencil or a fine point drawing pen); place them on the low temperature hot plate, which now has been reset to approximately 275° F.

9 Place a drop of mounting resin on the microscope slides and allow the solvent to evaporate.

10 When the incineration of the material on the cover glasses is complete, transfer the cover glasses, while still hot, to the mounting medium.

11 Allow the resin to penetrate the frustules (1-2 minutes).

12 Remove the slide, place it on a cool desk top, and press the cover glass lightly with a pencil eraser for a few seconds. The medium will harden in 5-10 seconds.

13 Scrape off the excess resin with a razor blade.

D The preparation is now ready for examination under an oil immersion objective.

ACKNOWLEDGEMENT:

Certain portions of this outline contains training material from a prior outline by M.E. Bender.

This outline was prepared by Dr. C.I. Weber, Chief, Biological Methods Section, Analytical Quality Control Laboratory, 1014 Broadway, Cincinnati, OH 45202.

LABORATORY: IDENTIFICATION OF ANIMAL PLANKTON

I INTRODUCTION

A The great majority of organisms commonly encountered in plankton analysis work are plants or at least plant-like (holophytic). Animals, however, (holozoic or nonchlorophyll bearing forms) are an important part of the community, and the ability to recognize them may be quite important.

B Many animals are soft bodied and so are best observed in the living condition, as they shrink and become otherwise distorted on preservation. There are consequently many which will not be available in a suitable form for the following exercise. Only such forms will be dealt with as can readily be obtained alive, or which retain essential characteristics on preservation.

II OBJECTIVES

A To Study the nature and use of a key for identifying organisms

B To Introduce the Beginners to the Use of the Microscope

C To Learn to Recognize Basic Animal Types

D To Identify Animal Plankton Species as Available, and to Become Familiar with the Literature

III PROCEDURE

A The Use of the Biological Key

1 Obtain a "Basic Invertebrate Collection" from the instructor.

2 Select a specimen designated by the instructor, and turn to the "Key to Selected Larger Groups of Aquatic Animals."

a Examine your specimen carefully, then read the first couplet of statements in the key (1a and 1b).

b Since the specimen is large enough to see, it obviously could not be the object of statement 1a. Therefore due to the nature of the key (as explained in the second paragraph of the introduction) the second alternative (1b) must apply. This alternative instructs us to proceed to couplet 2.

c From here on, follow from couplet to couplet, considering each couplet by itself, until a final selection leads to a name. If this name or couplet is, followed by another couplet number, this means that the group named is further subdivided.

3 Identify the other specimens in the Basic Invertebrate Collection in the same way.

4 Carry the identification further, to genus and species if possible, in one or more of the more detailed keys listed at the end of the "Key to Selected Larger Groups of Aquatic Animals."

B The Use of the Microscope

1 Obtain preliminary information from the instructor as to how to set up and operate the instrument.

2 Place a prepared slide of a printed letter on the stage and observe it successively under low (100X) and high (45X) powers. When the letter is right side up to you, how does it appear through the microscope?

3 Place a prepared slide of a micro-crustacean on the stage and identify it using the "Key to Selected Larger Groups of Aquatic Animals." Continue your

identification as far as possible using Eddy and Hodson's "Taxonomic Keys."

4 Prepare a "wet mount" under the direction of the instructor and identify the organism. Confirm your identification in one or more of the technical reference books available.

C Identify each of the specimens in the reference collection as to phylum and class, and then genus and species if possible (do not spend undue time on the species without assistance).

Make a flash card sketch of at least one organism of each phylum observed as an example of a type.

D Examine the living material provided. Sketch and identify animal forms encountered as far as time permits. Can you draw any conclusions as to the types of animal life found in the various habitats indicated?

This cutline was prepared by H. W. Jackson, Chief Biologist, National Training Center, DTTB, MDS, OWP, EPA, Cincinnati, OH 45226.

TECHNIQUES OF PLANKTON SAMPLING PROGRAMS

I INTRODUCTION

A A plan is necessary. "If you fail to plan, you are planning to fail." Overall objectives, integration with other survey units, statistical design.

B A planned program of plankton analysis should involve periodic sampling at weekly intervals or more often.

1 Most interference organisms are small, and hence have relatively short-life histories.

2 Populations of such organisms may fluctuate rapidly in response to changing water, weather, or seasons.

3 Seasonal growth patterns of plankton tend to repeat themselves from year to year, thus they are relatively predictable.

C A well-planned study or analysis of the growth pattern of plankton in one year will provide a basis for predicting conditions the following year.

1 Since the seasons and the years differ, the more records are accumulated, the more useful can they become.

2 As the time for an anticipated bloom of some trouble maker approaches, the frequency of analysis may be increased.

D Detection of a bloom in its early stages will facilitate more economical control.

II FIELD ASPECTS OF THE ANALYSIS PROGRAM

A Two general aspects of sampling are commonly recognized: quantitative and qualitative.

1 Qualitative examination tells what is present.

2 Quantitative tells how much.

3 Either approach is useful, a combination is best.

B Equipment of collecting samples in the field is varied.

1 A half-liter bottle will suffice for surface samples of phytoplankton if carefully taken. If zooplankton also are of interest, 2 or more liters should be collected. (See below).

2 Plankton nets concentrate the sample in the act of collecting, and capture certain larger forms which escape from the bottles. Only the more elaborate types are quantitative however.

3 A kemmerer-type sampler is suggested for depth samples.

4 Other methods such as the Clark-Bumpus sampler or the Juday plankton trap may be employed for special purposes.

C The location of sampling points is important

1 Both shallow and deep samples are suggested.

a "Shallow" samples should be taken at a depth of 6 inches to one foot.

b "Deep" samples should be taken at such intervals as the depth of the reservoir permits. There should be at least one open water sampling point.

c Each major bay or shoal area should have at least one sampling point.

d Additional sampling stations should be established on the basis of experience and resources.

e Samples may be composited if necessary to give an overall summary of conditions. Such summaries are not advised and should be interpreted with care.

A standardized vertical haul however, can be useful for routine comparisons.

III FACTORS WHICH INCLUENCE SAMPLING AND DATA COLLECTION

A Physical Features

1 Temperature

a Lakes are warmed in spring principally by the action of wind forcing the warmer water down into the cooler water against the forces of gravity.

b Thermal stratification

2 Turbidity

3 Color

4 Water movement

5 Light penetration

a A factor of turbidity, color, biological activity, and time of day

(1) Effective length of daylight dimenishes with the depth of the lake.

6 Wind velocity and direction

7 Bottom materials

8 Size, shape, and slope of lake basin

B Chemical Factors

1 Alkalinity, pH, and dissolved minerals excluding nitrogen and phosphorus

2 Dissolved oxygen

a From photosynthesis in sunlight

b From contact of lake surface with the air

c Fluctuates seasonally because of temperature and biological activity, and diurnally because of biological activity.

3 Nutrients for biological growth - especially nitrogen and phosphorus

a A given body of water will produce a given quanity of aquatic life. Biological production is determined primarily by the nutrients in solution in the water, and an increase in basic fertility will increase biological activity.

b Basic suppliers of nutrients include tributary streams, precipitation from the atmosphere, and interchange with lake bottom sediments.

IV FIELD PRESERVATION OF SAMPLES

Provision should be made for the field stabilization of the sample until the laboratory examination can be made. Techniques and materials are listed below. No "ideal" preservative or technique has yet been developed each has its virtues.

A Refrigeration or icing. The container containing the sample can be cooled, but under no circumstances should ice be dropped into the sample.

B Preservation by 3-5% formaline is time-tested and widely used. Formaline shrinks animal tissue, fades colors, and makes all forms brittle.

C Ultra-violet sterilization is useful in the laboratory to retard decomposition of plankton.

D Lugol's solution is often used.

E A special merthiolate preservative developed by the FWPCA Water Pollution Surveillance System which has proved very satisfactory and is described in reference No. 9.

V SUMMARY AND CONCLUSIONS

A The field sampling program should be carefully planned to evaluate all significant locations in the reservoir or stream, giving due consideration to the capacity of the laboratory.

B Adequate records and notes should be made of field conditions and associated with the laboratory analyses in a permanent file.

C Once a procedure for processing plankton is adopted, it should be used exclusively by all workers at the plant.

D Such a procedure should enable the water plant operator to prevent plankton troubles or at least to anticipate them and have corrective materials or equipment stockpiled.

ACKNOWLEDGMENT:

Portions of this outline were prepared by K. M. Mackenthun, Biologist, formerly with Technical Advisory and Investigation Activities, FWPCA, SEC, Cincinnati, Ohio.

REFERENCES

1 APHA. Standard Methods for the Examination of Water and Wastewater. 13th Ed, pp 726-742, NY, 1971.

2 Hutcheson, George E. A Treatise on Limnology. John Wiley and Co. New York. 1957.

3 Jackson, H. W. Biological Examination (of plankton) Part III in Simplified Procedures for Water Examination. AWWA Manual M12. Am. Water Works Assoc., N.Y. 1964.

4 Lackey, J. B. The Manipulation and Counting of River Plankton and Changes in Some Organisms Due to Formalin Preservation. Public Health Reports, 53: 2080-93. 1938.

5 Mackenthun, K. M., Ingram, W. M., and Ralph Porges. Limnological Aspects of Recreation Lakes, DHEW, PHS Publication No. 1167, 1964.

6 Olson, Theodore A. and Burgess, Frederick J. Pollution and Marine Ecology. Interscience Publishers. 364 pp. 1967.

7 Palmer, C. M. Algae in Water Supplies. U. S. Department of Health, Education, and Welfare, Public Health Service Publication No. 657, Superintendent of Documents, Washington 25, D.C.

8 Schwoerbel, J. Methods of Hydrobiology (Freshwater Biology). Pergamon Press, 1970.

9 Weber, C.I. Methods of Collection and Analysis of Plankton and Periphyton Samples in the Water Pollution Surveillance System. App. and Devel. Rep. (AQC Lab., 1014 Broadway, Cincinnati, OH 45202) 19 pp 1966.

10 Welch, P.S. Limnological Methods. The Blakiston Co., Phila. Toronto. 1948.

11 Williams, L. G. Plankton Population Dynamics, in National Water Quality Network, Supplement 2, U.S. PHS Pub. No. 663. 1962.

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, DTTB, MDS, OWP, EPA, Cincinnati, OH 45268.

PREPARATION AND ENUMERATION OF PLANKTON IN THE LABORATORY

I RECEPTION AND PREPARATION OF SAMPLES

A Preliminary sampling and analysis is an essential preliminary to the establishment of a permanent or semi-permanent program.

B Concentration or sedimentation of preserved samples may precede analysis.

1 Batch centrifuge

2 Continuous centrifuge

3 Sedimentation

C Unpreserved (living) samples should be analyzed at once or refrigerated for future analysis.

II PREPARATION OF MERTHIOLATE PRESERVATIVE

A The Water Pollution Surveillance System of the FWPCA has developed a modified merthiolate preservative. (Williams, 1967) Sufficient stock to make an approximately 3.5% solution in the bottle when filled is placed in the sample bottle in the laboratory. The bottle is then filled with water in the field and returned to the laboratory for analysis.

B Preparation of Merthiolate Preservative

1 Merthiolate is available from many chemical laboratory supply houses; one should specify the water soluble sodium salt.

2 Merthiolate stock: dissolve approximately 1.5 gram of sodium borate (borax and approximately 1 gram of merthiolate in 1 liter of distilled water.

The amount of sodium borate and merthiolate may be varied slightly to adjust to different waters, climates, and organic contents.

3 Prepare a saturated aqueous Lugol's solution as follows:

a Add 60 grams of potassium iodide (KI) and 40 grams of iodine crystals to 1 liter of distilled water.

4 Prepare the preservative solution by adding approximately 1.0 ml of the Lugol's solution to 1 liter of merthiolate stock.

III SAMPLE ANALYSIS

A Microscopic examination is most frequently employed in the laboratory to determine what plankton organisms are present and how many there are:

1 Optical equipment need not be elaborate but should include.

a Compound microscope with the following equipment:

1) Mechanical stage
2) Ocular: 10X, with Whipple type counting eyepiece or reticule
3) Objectives:
    approx. 10X(16mm)
    approx. 20X(8 mm)
    approx. 40X(4 mm)
    approx. 95X(1.8 mm)(optional)

A 40X objective with a working distance of 12.8 mm and an erect image may be obtained as special equipment. A water immersion objective (in addition to oil) might be considered for use with water mounts.

Binocular eyepieces are optional.

Stage micrometer (this may be borrowed, if necessary, as it is usually used only once, when the equipment is calibrated)

b Inverted microscopes offer certain advantages but are not widely available. The same is true of some of the newer optical systems such as phase contrast microscopy. These are often excellent but expensive for routine plant use.

2 Precision made counting chambers are required for quantitative work with liquid mounts.

a Sedgwick-Rafter cells (hereafter referred to as S-R cell) are used for routine counts of medium and larger forms.

b Extremely small forms or "nannoplankton" may be counted by use of the nannoplankton (or Palmer) cell, a Fisher-Littman cell, a hemacytometer, the Lackey drop method, or by use of an inverted microscope.

3 Previous to starting serious analytical work, the microscope should be calibrated as described elsewhere. Dimensions of the S-R cell should also be checked, especially the depth.

4 Automatic particle counters may be useful for coccoid organisms.

B Quantitative Plankton Counts

1 All quantitative counting techniques involve the filling of a standard cell of known dimensions with either straight sample or a concentrate or dilution thereof.

2 The organisms in a predetermined number of microscope fields or other known area are then observed, and by means of a suitable series of multiplier factors, projected to a number or quantity per ml gallon, etc.

3 Direct counting of the unconcentrated sample eliminates manipulation, saves time, and reduces error. If frequency of organisms is low, more area may need to be examined or concentration of the sample may be in order.

4 Conventional techniques employing concentration of the sample provide more organisms for observation, but because they involve more manipulations, introduce additional errors and take more time.

C Several methods of counting plankton are in general use.

1 The numerical or clump count is regarded as the simplest.

a Every organism observed must be enumerated. If it cannot be identified, assign a symbol or number and make a sketch of it on the back of the record sheet.

b Filaments, colonies and other associations of cells are counted as units, equal to single isolated cells. Their identity as indicated on the record sheet is the key to the significance of such a count.

2 Individual cell count. In this method, every cell of every colony or clump of organisms is counted, as well as each individual single-celled organism.

3 The areal standard unit method offers certain technical advantages, but also involves certain inherrent difficulties.

a An areal standard unit is 400 square microns. This is the area of one of the smallest subdivided squares in the center of the Shipple eyepiece at a magnification of 100X.

b In operation, the number of areal units of each species is recorded on the record sheet rather than the number of individuals. Average areas of the common species are

are sometimes printed on record sheets for a particular plant to obviate the necessity of estimating the area of each cell observed individually.

c The advantage of the method lies in the cognizance taken of the relative masses of the various species as indicated by the area presented to the viewer. These areas, however, are often very difficult to estimate.

4 The cubic standard unit method is a logical extension of the areal method, but has achieved less acceptance.

5 Separate field count

a In counting separate fields, the question always arises as to how to count organisms touching or crossed by the edge of the Whipple field. Some workers estimate the proportion of the organism lying inside the field as compared to that outside. Only those which are over half way inside are counted.

b Another system is to select two adjacent sides of the square for reference, such as the top and left boundaries. Organisms touching these lines in any degree, from outside or inside, are then counted, while organisms touching the opposite sides are ignored. It is important to adopt some such system and adhere to it consistently.

c It is suggested that if separate microscopic fields are examined, a standard number of ten be adopted. These should be evenly spaced in two rows about one-third of the distance down from the top and one-third of the distance up from the bottom of the S-R cell.

6 Multiple area count. This is an extension of the separate field count. A considerable increase in accuracy has recently been shown to accrue by emptying and refilling the S-R cell, after each group of fields are counted and making up to 5 additional such counts. This may not be practical with high counts.

7 The strip count. When a rectangular slide such as the S-R cell is used, a strip (or strips) the entire length (or known portion thereof) of the cell may be counted instead of separate isolated fields. Marking the bottom of the cell by evenly spaced cross lines as explained elsewhere greatly facilitates counting.

a When the count obtained is multiplied by the ratio of the width of the strip counted to the width of the cell, the product is the estimated number of organisms in the cell, or per ml.

b When the material in the cell is unconcentrated sample water, this count represents the condition of the water being evaluated without further calculation.

8 Survey count. A survey count is an examination of the entire area of a volumetric cell using a wide field low power microscope. The objective is to locate and record the larger forms, especially zooplankton such as copepods or large rotifers which may be present in size. Special large capacity cells are often employed for this purpose. For still larger marine forms, numerous special devices have been created.

9 Once a procedure for concentration and/or counting is adopted by a plant or other organization it should be used consistently from then on so that results from year to year can be compared.

D Differential or qualitative "counts" are essentially lists of the kinds of organisms found.

E Proportional or relative counts of special groups are often very useful. For example, diatoms. It is best to always count a standard numbers of cells.

F Plankton are sometimes measured by means other than microscopic counts.

1 Settled volume of killed plankton in an Imhoff cone may be observed after a standard length of time. This will evaluate primarily only the larger forms.

2 A gravimetric method employs drying at 60° C for 24 hours followed by ashing at 600° C for 30 minutes. This is particularly useful for chemical and radiochemical analysis.

3 Chemical and physical evaluation of plankton populations employing various instrumental techniques are coming to be widely used. Both biomass and productivity rates can be measured. Such determinations probably achieve their greatest utility when coordinated with microscopic examination.

4 The membrane (molecular) filter has a great potential, but a generally acceptable technique has yet to be perfected.

a Bacteriological techniques for coliform determination are widely accepted.

b Nematodes and larger organisms can readily be washed off of the membrane after filtration.

c It is also being used to measure ultraplankton that pass treatment plant operations.

d Membranes can be cleared and organisms deposited thereon observed directly, although accessory staining is desirable.

e Difficulties include a predilection of extremely fine membranes to clog rapidly with silt or increase in plankton counts, and the difficulty of making observations on individual cells when the organisms are piled on top of each other. It is sometimes necessary to dilute a sample to obtain suitable distribution.

IV SUMMARY AND CONCLUSIONS

A The field sampling program should be carefully planned to evaluate all significant locations in the reservoir or stream, giving due consideration to the capacity of the laboratory.

B Adequate records and notes should be made of field conditions and associated with the laboratory analyses in a permanent file.

C Optical equipment in the laboratory should be calibrated.

D Once a procedure for processing plankton is adopted, it should be used exclusively by all workers at the plant.

E Such a procedure should enable the water plant operator to prevent plankton troubles or at least to anticipate them and have corrective materials or equipment stockpiled.

REFERENCES

1 Ely Lilly Company. Merthiolate as a Preservative. Ely Lilly & Co. Indianapolis 6, Indiana.

2 Gardiner, A.C. Measurement of Phytoplankton Population by the Pigment Extraction Method. Jour. Marine Biol. Assoc. 25(4):739-744. 1943.

3 Goldberg, E.D., Baker, M., and Fox, D.L. Microfiltration in Oceanographic Research Sears Foundation. Jour. Mar. Res. 11:194-204. 1952.

4 Ingram, W. M., and Palmer, C. M. Simplified Procedures for Collecting, Examining, and Recording Plankton in Water. Jour. AWWA. 44(7): 617-624. 1952.

5 Jackson, H. W. Bilogical Examination (of plankton) Part III in Simplified Procedures for Water Examination. AWWA Manual M 12. Am. Water Works Assoc. N.Y. 1964.

6 Lund, J. W. G., and Talling, J. F. Botanical Limnological Methods with Special References to the Algae. Botanical Review. 23(8&9):489-583. October, 1957.

7 Weber, C. I. The Preservation of Plankton Grab Samples. Water Pollution Surveillance Systems, Applications and Development Report No. 26, USDI, FWPCA, Cincinnati, Ohio. (1967)

8 Williams, L. G. Plankton Population Dynamics. National Water Quality Network Supplement 2. U. S. Public Health Service Publ. No. 663. (1962)

9 Wohlschag, D. D., and Hasler, A. D. Some Quantitative Aspects of Algal Growth in Lake Mendota. Ecology. 32(4):581-593. (1951)

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CALIBRATION AND USE OF PLANKTON COUNTING EQUIPMENT

I INTRODUCTION

A With the exception of factory-set instruments, no two microscopes can be counted upon to provide exactly the same magnification with any given combination of oculars and objectives. For accurate quantitative studies, it is therefore necessary to standardize or "calibrate" each instrument against a known standard scale. One scale frequently used is a microscope slide on which two millimeters are subdivided into tenths, and two additional tenths are subdivided into hundredths. Figure 3.

B In order to provide an accurate measuring device in the microscope, a Whipple Plankton Counting Square or reticule (Figure 2a) is installed in one ocular (there are many different types of reticules). This square is theoretically of such a size that with a 10X objective, a 10X ocular, and a tube length of 160 mm, the image of the square covers a square area on the slide one mm on a slide. Since this objective is rarely attained however, most microscopes must be standardized or "calibrated" as described below in order to ascertain the actual size of the Whipple Square as seen through the microscope (hereinafter referred to as the "Whipple field"). This process is schematically represented in Figures 5 and 7. If the Whipple eyepiece is to be used at more than one magnification, it must be recalibrated for each. A basic type of monocular microscope is shown in Figure 1.

C Microscopes with two eyepieces (binocular) are a convenience but not essential. Like modern cars they are not only great "performers," but also complicated to service or, in this instance, calibrate. On some instruments, changing the interpupillary distance also changes the tube length, on others it does not. The "zoom" feature on certain scopes is also essentially a system for changing the tube length.

The resultant is that in addition to calibration at each combination of eyepiece and objective, any other factor which may affect magnification must also be considered. In some instances this may mean setting up a table of calibrations at a series of microscope settings. Another procedure is to select a value for each of the variables involved (interpupillary distance, zoom, etc.) and calibrate the scope at that combination. Then each time the scope is to be used for quantitative work, re-set each variable to the value selected. A separate multiplication factor must be calculated for each adjustment which changes the magnification of the instrument.

Since the Whipple Square can be used to measure both linear dimensions and square areas, both should be recorded on an appropriate form. A suggested format is shown in Figure 6.

(Data written in are used as an illustration and are not intended to apply to any particular microscope. An unused form is included as Figure 6-A.)

II THE CALIBRATION PROCEDURE

A Installing the Whipple Square or Reticule

To install the reticule in the ocular (usually the right one on a binocular microscope), carefully unscrew the upper lens mounting and place the reticule on the circular diaphragm or shelf which will be found approximately half way down inside (Figure 4). Replace the lens mounting and observe the markings on the reticule. If they are not in sharp focus, remove and turn the reticule over.

On reticules with the markings etched on one side of a glass disc, the etched surface can usually be recognized by shining the disc at the proper angle in a light. The markings will usually be in the best focus with the etched surface down. If the markings are sandwiched between two glass discs cemented together, both sides are alike, and the focus may not be quite as sharp.

B Observation of the Stage Micrometer

Replace the ocular in the microscope and observe the stage micrometer as is illustrated schematically in Figure 5: Calibration of the Whipple Square. On a suitably ruled form such as the one illustrated, Figure 6, Calibration Data, record the actual distance in millimeters subtended by the image of the entire Whipple field and also by each of its subdivisions. This should be determined for each significant settling of the interpupillary distance for a binocular microscope, and also for each combination of lenses employed. Since oculars and objectives marked with identical magnification, and since microscope frames too may differ, the serial or other identifying number of those actually calibrated should be recorded. It is thus apparent that the determinations recorded will only be valid when used with the lenses listed and on that particular microscope.

C Use of the 20X Objective

Due to the short working distance beneath a 46X (4mm) objective, it is impossible to focus to the bottom of the Sedgewick-Rafter plankton counting cell with this lens. A 10X (16mm) lens on the other hand "wastes" space between the front of the lens and the coverglass, even when focused on the bottom of the cell. In order to make the most efficient use possible of this cell then, an objective of intermediate focal length is desirable. A lens with a focal length of approximately 8 mm, having a magnification of 20 or 21X will meet these requirements. Such lenses are available from American manufacturers and are recommended for this type of work.

III CHECKING THE CELL

The internal dimensions of a Sedgewick-Rafter plankton counting cell should be 50 mm long by 20 mm wide by 1 mm deep (Figure 8).

The actual horizontal dimensions of each new cell should be checked with calipers, and the depth of the cell checked at several points around the edge using the vertical focusing scale engraved on the fine adjustment knob of most microscopes. One complete rotation of the knob usually raises or lowers the objective 1 mm or 100 microns (and each single mark equals 1 micron). Thus, approximately ten turns of the fine adjustment knob should raise the focus from the bottom of the cell to the underside of a coverglass resting on the rim. Make these measurements on an empty cell. The use of a No. 1 or 1-1/2, 24 × 60 mm coverglass is recommended rather than the heavy coverglass that comes with the S-R cell, as the thinner glass will somewhat conform to any irregularities of the cell rim (hence, also making a tighter seal and reducing evaporation when in actual use). Do not attempt to focus on the upper surface of the rim of an empty cell for the above depth measurements, as the coverglass is supported by the highest points of the rim only, which are very difficult to identify. Use the average of all depth measurements as the "true" depth of the cell. To simplify calculations below, it will be assumed that we are dealing with a cell with an average depth of exactly 1.0 mm.

IV PROCEDURE FOR STRIP COUNTS USING THE SEDGEWICK-RAFTER CELL

A Principles

Since the total area of the cell is 1000 mm^2, the total volume is 1000 mm^3 or 1 ml. A "strip" the length of the cell thus constitutes a volume (V_1) 50 mm long, 1 mm deep, and the width of the Whipple field.

The volume of such a strip in mm^3 is:

$$\begin{aligned} V_1 &= 50 \times \text{width of field} \times \text{depth} \\ &= 50 \times w \times 1 \\ &= 50\,w \end{aligned}$$

In the example given below on the plate entitled Calibration Data, at a magnification of approximately 200X with an interpupillary setting of "60", the width of the Whipple field is recorded as approximately 0.55 mm (or 550 microns). In this case, the volume of the strip is:

$$V_1 = 50\,w = 50 \times 0.55 = 27.5\ (mm^3)$$

B Calculation of Multiplier Factor

In order to convert plankton counts per strip to counts per ml, it is simply necessary to multiply the count obtained by a factor (F_1) which represents the number of times the volume of the strip examined (V_1) would be contained in 1 ml or 1000 mm^3. Thus in the example given above:

$$\begin{aligned} F_1 &= \frac{\text{volume of cell in } mm^3}{\text{volume examined in } mm^3} \\ &= \frac{1000}{V} = \frac{1000}{27.5} = 36.36 \\ &= \text{approx. } 36 \end{aligned}$$

If more than one strip is to be counted, the factor for two, three, etc., strips could be calculated separately using the same relationships outlined above, changing only the measurement for the length of

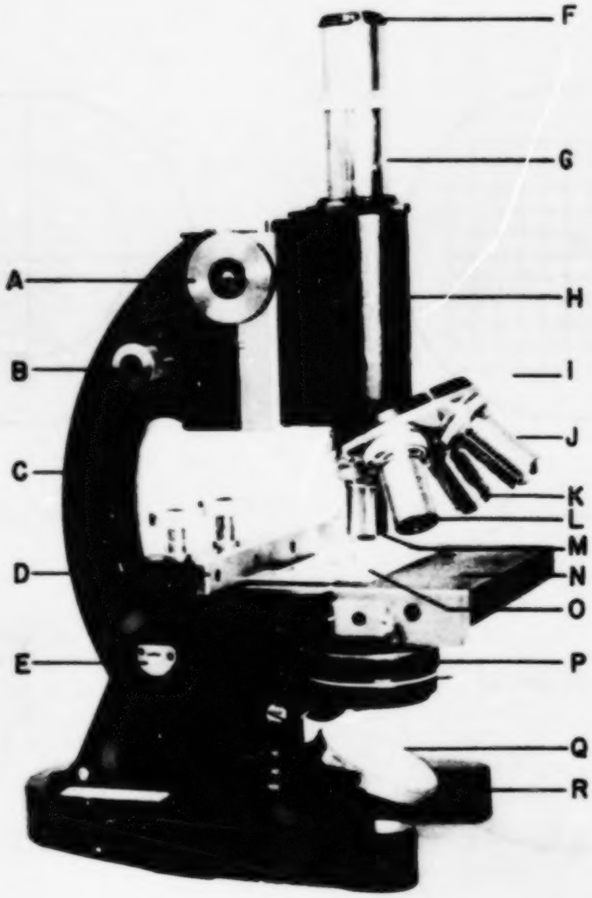


Figure 1. THE COMPOUND MICROSCOPE

A) coarse adjustment; B) fine adjustment; C) arm or pillar; D) mechanical stage which holds slides and is movable in two directions by means of the two knobs; E) pivot or joint. This should not be used or "broken" while counting plankton; F) eyepiece (or ocular cf: figure 4); G) draw tube. This will be found on monocular microscopes only (those having only one eyepiece). Adjustment of this tube is very helpful in calibrating the microscope for quantitative counting (Sec. 5.5.2.2.). H) body tube. In some makes of microscopes this can be replaced with a body tube having two eyepieces, thus making the 'scope into a "binocular." I) revolving nosepiece on which the objectives are mounted; J) through M are objectives, any one of which can be turned toward the object being studied. In this case J is a 40X, K is a 100X, L is a 20X, and M is a 10X objective. The product of the magnification power of the objective being used times the magnification power of the eyepiece gives the total magnification of the microscope. Different makes of microscopes employ objectives of slightly different powers, but all are approximately equivalent. N) stage of the microscope; O) Sedgwick-Rafter cell in place for observation; P) substage condenser; Q) mirror; R) base or stand; note: for information on the optical system, consult reference 3. (Photo by Don Moran.).

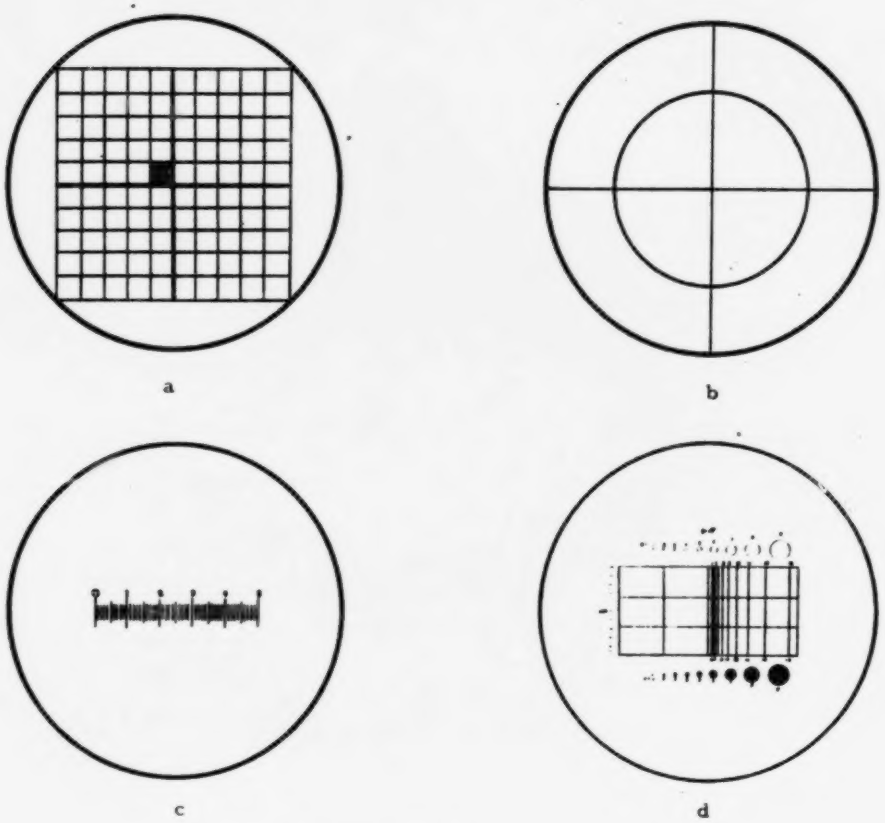


Figure 2

Types of eyepiece micrometer discs or reticules (reticules, graticules, etc.). When dimensions are mentioned in the following description, they refer to the markings on the reticule discs and not to the measurements subtended on the microscope slide. The latter must be determined by calibration procedures such as those described elsewhere. (a) Whipple plankton counting eyepiece. The fine rulings in the subdivided square are sometimes extended to the margin of the large square to facilitate the estimation of sizes of organisms in different parts of the field. (b) Quadrant ruling with 8.0 mm circle, for counting bacteria in milk smears for example. (c) Linear scale 5.0 mm divided into tenths. For measurement of linear dimensions. (d) Porton reticule for estimating the size of particles. The sizes of the series of discs is based on the square root of two so that the areas of successive discs double as they progress in size.

strip counted. Thus for two strips in the example cited above:

$$V_2 = 100W = 100 \times 0.55 = 55 \text{ mm}^3$$

$$F_2 = \frac{1000}{V_1} = \frac{1000}{55} = 18.2$$

It will however be noted that $F_2 = \frac{F_1}{2}$.

Likewise a factor F_3 for three strips would equal $\frac{F_1}{3}$ or approximately 12, etc.

C An Empirical "Step-Off" Method

A simpler but more empirical procedure for determining the factor is to consider that if a strip 20 mm wide were to be counted the length of the cell, that the entire 1000 mm^3 would be included since the cell is 20 mm wide and 1 mm deep.

This 20 mm strip width can be equated to 1000 mm^3. If a strip (or the total of 2 or more strips) is less than 20 mm in width, the quotient of 20 divided by this width will be a multiplier factor for converting from count per strip(s) to count per ml.

Thus in the example cited above where at an approximate magnification of 200X and with an interpupillary setting of 60, the width of the Whipple field is .55 mm. Then:

$$F_1 = \frac{20}{.55} = 36.36 \text{ or approx. } 36$$

(as above)

If two strips are counted:

$$\begin{array}{r} .55 \\ +\ .55 \\ \hline 1.10 \end{array} \text{ and } F_2 = \frac{20}{1.1} = 18.2 = \text{approx. } 18, \text{etc.}$$

This same value could be obtained without the use of a stage micrometer by carefully moving the cell sidewise across the field of vision by the use of a mechanical stage. Count the number of Whipple fields in the width of the cell. There should be approximately 36 in the instance cited above.

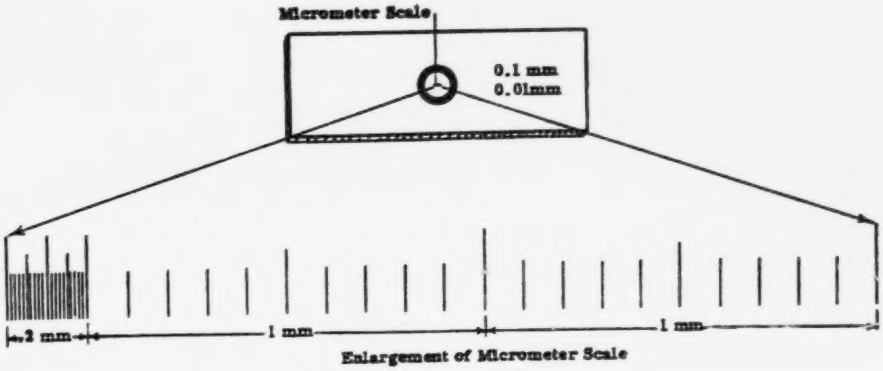
V SEPARATE FIELD COUNT USING THE SEDGEWICK-RAFTER CELL

A Circumstances of Use

The use of concentrated samples, local established programs, or other circumstances

Figure 3. STAGE MICROMETER

The type illustrated has two millimeters divided into tenths, plus two additional tenths subdivided into hundredths.



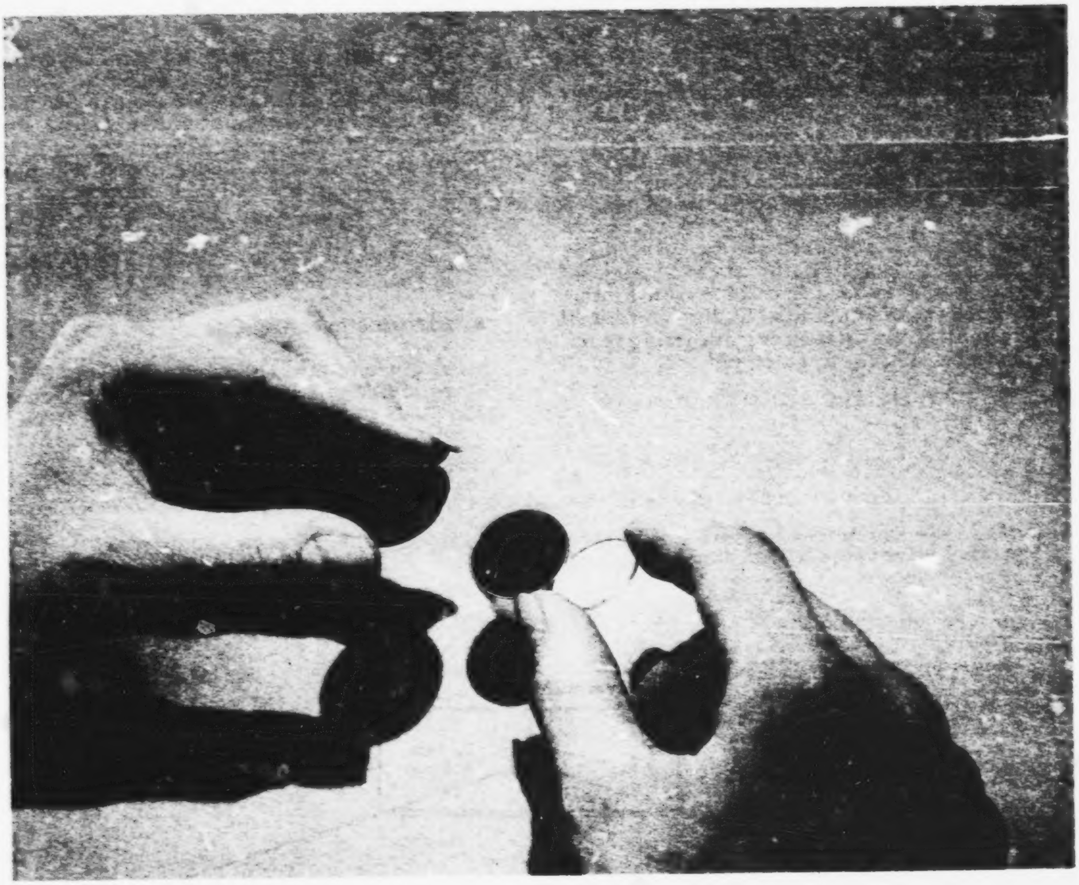


Figure 4. Method of Mounting the Whipple Disc in an Ocular. Note the upper lens of the ocular which has been carefully unscrewed, held in the left hand, and the Whipple disc, held in the right hand. (Photo by Don Moran).

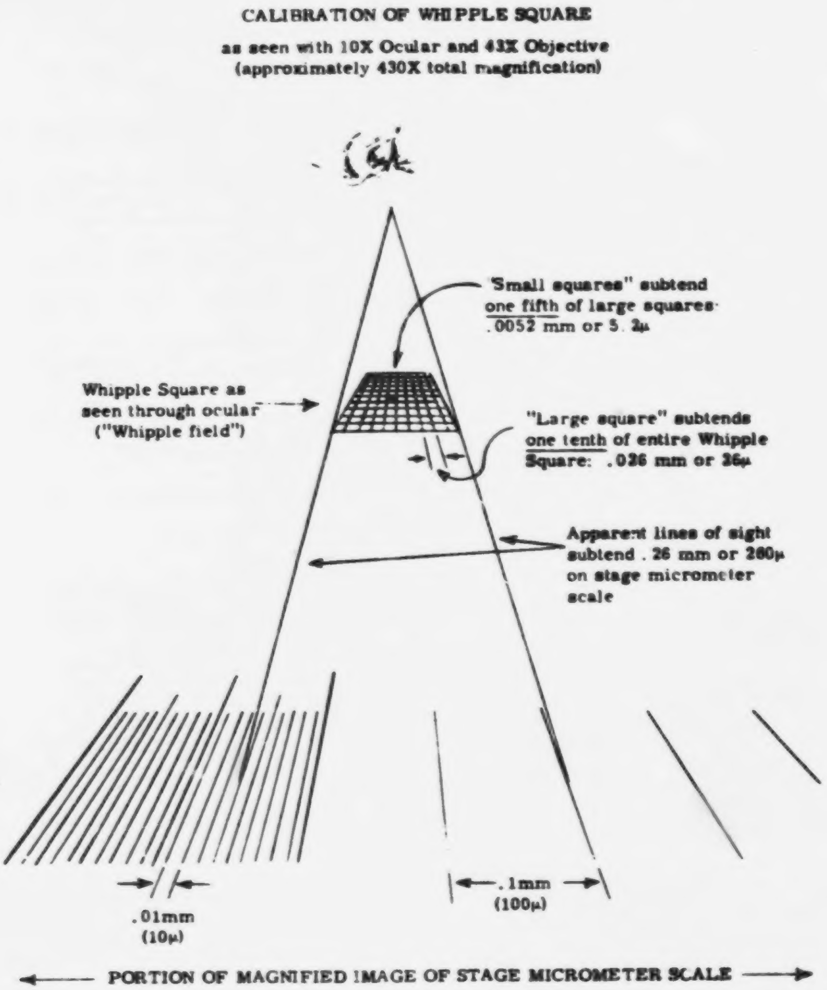

CALIBRATION OF WHIPPLE SQUARE
as seen with 10X Ocular and 43X Objective
(approximately 430X total magnification)
"Small squares" subtend one fifth of large squares .0052 mm or 5.2µ
Whipple Square as seen through ocular ("Whipple field")
"Large square" subtends one tenth of entire Whipple Square: .026 mm or 26µ
Apparent lines of sight subtend .26 mm or 260µ on stage micrometer scale
.01mm (10µ)
.1mm (100µ)
PORTION OF MAGNIFIED IMAGE OF STAGE MICROMETER SCALE

Figure 5

CALIBRATION OF THE WHIPPLE SQUARE

The apparent relationship of the Whipple Square is shown as it is viewed through a microscope while looking at a stage micrometer with a magnification of approximately 430X (10X ocular and 43X objective).

MICROSCOPE CALIBRATION DATA

Microscope No. 62379

Approximate Magnification	Tube Length, or Interpupillary Setting	Linear dimensions of Whipple squares in millimeters*			Factor for Conversion to count/ml
		Whole	Large	Small	
100X, obtained with					(2 S-R Strips)
Objective Serial No.					
476421(10x)	50	1.130	0.113	0.0226	8.9
and Ocular Serial No	60	1.115	0.111	0.0222	9.0
1296742(10x)	70	1.100	0.110	0.0222	9.1
200X, obtained with					(2 S-R Strips)
Objective Serial No.					
6149289(21x)	50	0.560	0.056	0.0112	17.9
and Ocular Serial No.	60	0.550	0.055	0.0110	18.2
1296742(10x)	70	0.545	0.054	0.0109	18.3
400X, obtained with					(Nannoplankton) (cell-20 fields)
Objective Serial No.					
259184(43x)	50	0.267	0.0267	.0053	1724.
and Ocular Serial No.	60	0.263	0.0263	.0053	1786.
1296742(10x)	70	0.260	0.0260	.0052	1852.

*1 mm = 1000 microns

Microscope calibration data. The form shown is suggested for the recording of data pertaining to a particular microscope. Headings could be modified to suit local situations. For example, "Interpupillary Setting" could be replaced by "Tube Length" or the "2S-R Strips" could be replaced by "per field" or "per 10 fields."

Figure 6

MICROSCOPE CALIBRATION DATA

Microscope No. ______________

Approximate Magnification	Tube Length, or Interpupillary Setting	Linear dimensions of Whipple squares in millimeters*			Factor for Conversion to count/ml
		Whole	Large	Small	
100X, obtained with					**(2 S-R Strips)**
Objective Serial No.					
_________					
and Ocular Serial No.					
_________					
200X, obtained with					**(2 S-R Strips)**
Objective Serial No.					
_________					
and Ocular Serial No.					
_________					
400X, obtained with					**(Nannoplankton) (cell-20 fields)**
Objective Serial No.					
_________					
and Ocular Serial No.					
_________					

*1mm = 1000 microns

BI. AQ. pl. 8. 10. 60.

Figure 6-A

MICROSCOPE CALIBRATION DATA

Suggested work sheet for the calibration of a microscope. Details will need to be adapted to the particular instrument and situation.

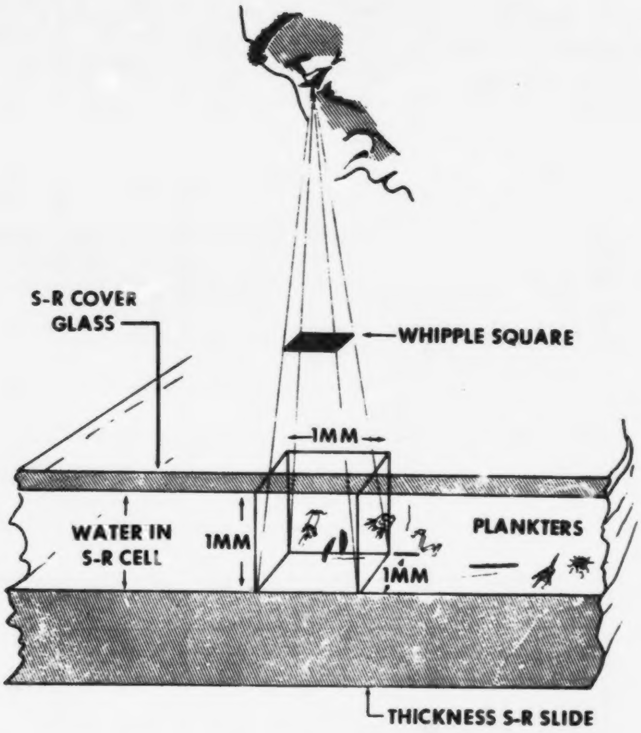

S-R COVER GLASS
WHIPPLE SQUARE
1MM
WATER IN S-R CELL
1MM
PLANKTERS
1MM
THICKNESS S-R SLIDE

Figure 7

A cube of water as seen through a Whipple square at 100X magnification in a Sedgewick-Rafter cell. The figure is drawn as if the microscope were focused on the bottom of the cell, making visible only those organisms lying on the bottom of the cell. The little "bug" (copepod) halfway up, and the algae filament at the top would be out of focus. The focus must be moved up and down in order to study (or count) the entire cube.

may make it necessary to employ the more conventional technique of counting one or more separate Whipple fields instead of the strip count method. The basic relationships outlined above still hold, namely:

$$F = \frac{\text{volume cell in mm}^3}{\text{volume examined in mm}^3}$$

B Principles Involved

The volume examined in this case will consist of one or more squares the dimensions of the Whipple field in area and 1 mm in depth (Figure 7). Common practice for routine work is to examine 10 fields, but exceptionally high or low counts or other circumstances may indicate that some other number of fields should be employed. In this case a "per field" factor may be determined to be subsequently divided by the number of fields examined as with the strip count. The following description however is based on an assumed count of 10 fields.

C Calculation of Multiplier Factor

As stated above, the total volume represented in the fields examined consists of the total area of the Whipple fields multiplied by the depth.

$$V_4 = \text{(side of Whipple field)}^2 \times \text{depth}$$

$$\text{(1 mm)} \times \text{no. of fields counted)}$$

For example, let us assume an approximate magnification of 100X (see Figures 6 and 7 and an interpupillary setting of "50". The observed length of one side of the Whipple field in this case is 1.13 mm. The calculation of V_4 is thus:

$$V_4 = \text{side}^2 \times \text{depth} \times \text{no. of fields}$$

$$= 1.13 \times 1.13 \times 1 \times 10 = 12.8 \text{ mm}^3$$

The multiplier factor is obtained as above (Section IV A):

$$F_4 = \frac{\text{volume cell in mm}^3}{\text{volume examined in mm}^3}$$

$$= \frac{1000}{12.8} = \text{(approx.) } 78$$

(If one field were counted, the factor would be 781, for 100 fields it would be 7.8.)

NANNOPLANKTON COUNTING

For counting nannoplankton using the high dry power (10X ocular and 43X objective) and the "nannoplankton counting cell" (Figure 9) which is 0.4 mm deep, a minimum of 20 separate Whipple fields is suggested. The same general relationships presented above (Section IV) can be used to obtain a multiplier or factor (F_5) to convert counts per 20 fields to counts per ml.

To take another example from Figure 4, at an approximate magnification of 400X and an interpupillary setting of 70 (see also Figure 3) we observe that one side of the Whipple field measures 0.260 mm. The volume of the fields examined is thus obtained as follows:

$$V_5 = \text{side}^2 \times \text{depth} \times \text{no. of fields}$$

$$= 0.26 \times 0.26 \times 0.4 \times 20 = .54 \text{ mm}^3$$

$$\text{and } F_5 = \frac{1000}{.54} = \text{(approx.) } 1850$$

It should be noted that the volume of the nannoplankton cell, .1 ml, is of no significance in this particular calculation.

REFERENCES

1 American Public Health Association, et. al. Standard Methods for the Examination of Water, Sewage, and Industrial Wastes. 13th Edition. Am. Public Health Assoc. New York. 1970.

2 Jackson, H.W. and Williams, L.G. The Calibration and Use of Certain Plankton Counting Equipment. Trans. Am. Mic. Soc. LXXXI(1):96-103. 1962.

3 Ingram, W. M. and Palmer, C. M. Simplified Procedures for Collecting, Examining, and Recording Plankton in Water. Jour. Am. Water Works. Assoc. 44(7): 617-724. 1952.

4 Palmer, C. M. Algae in Water Supplies. U. S. D. H. E. W. Public Health Service Pub. No. 657. 1959.

5 Palmer, C. M. and Maloney, T. E. A New Counting Slide for Nannoplankton. American Soc. Limnol. and Oceanog. Special Publications No. 21. pp. 1-6. 1954.

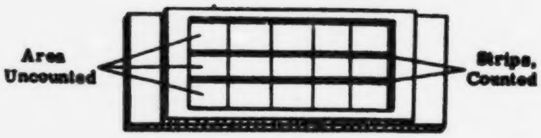


Figure 8

Sedgewick-Rafter counting cell showing bottom scored across for ease in counting strips. The "strips" as shown in the illustration simply represent the area counted, and are not marked on the slide. The conventional dimensions are 50 × 20 × 1 mm, but these should be checked for accurate work.

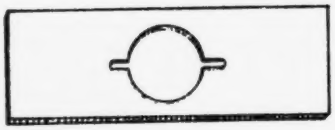


Figure 9

Nannoplankton cell. Dimensions of the circular part of the cell are 17.9 mm diameter × 0.4 mm depth. When covered with a coverglass, the volume contained is 0.1 ml. The channels for the introduction of sample and the release of air are 2 mm wide and approximately 5 mm long. This slide is designed to be used with the 4 mm or 43X (high dry) objective.

6 Welch, Paul S. Limnological Methods. Blakiston Company. Phila. Toronto. 1948.

7 Whipple, G. C., Fair, G. M., and Whipple, M. C. The Microscopy of Drinking Water. John Wiley and Sons. New York. 1948.

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, DTTB, MDS, OWP, EPA, Cincinnati, OH 45268.

DETERMINATION OF ODORS

I INTRODUCTION

Odor shall be determined substantially as prescribed by the 11th edition of "Standard Methods for the Examination of Water and Sewage", subject to certain stipulations and modifications made necessary by the International Joint Commission.

The procedure and technique to be followed are described below.

II REAGENTS AND APPARATUS

A Odor-free water - prepared by passing tap water through activated carbon at a slow rate of speed. Activated carbon can be placed at the bottom of a 20-liter glass bottle. The bottle can be connected to the tap by rubber tubing leading to glass tubing above the water. The outlet from the bottom of the bottle should be glass tubing. A trap made of inch glass tubing filled with activated carbon is placed at the end of the outlet.

B 500 ml glass-stoppered Erlenmeyer flasks, each flask with a number. Glassware must be thoroughly cleaned and rinsed several times with odor-free water before each use.

C Chemical Thermometer (0-100°C)

D 10 ml Mohr pipettes, 25 ml graduated cylinders, 50 ml graduated cylinders, 200 ml graduated cylinders, 500 ml graduated cylinders. Other pipettes and cylinders as needed.

E One liter glass-stoppered bottles to hold samples of water being examined. Other glass bottles and flasks as needed.

III PRECAUTIONS

A Certain conditions are required to obtain consistent results. Considerable practice with the test is desirable to develop consistent sensitivity to the sense of smell.

1 In view of the perishability of the odor test, these determinations should be made immediately after collection.

2 The prepared odor-free water should be truly free of all detectable odor.

3 All glassware must be free of odor. This is accomplished by thorough cleansing followed by several rinses with odor-free water.

4 All dilutions should be compared with an odorless standard. This aids the observer in deciding whether air odor is present or not.

5 All dilutions when examined for odor should be of a uniform temperature, deviation not to exceed 1°C.

6 A sudden change in the character of the odor during the testing procedure should be considered as a warning that there may be interference from outside odors or that the diluting water may not be odor-free. The character of odor should always be recorded for future consideration.

B To eliminate psychological influences, the samples should be coded and intermixed so as not to suggest to the observer what odor concentration is being observed.

1 Bottles should be colored or covered with odor-free material or the observer blindfolded to eliminate auto suggestion

since many samples may possess color or turbidity.

2 Test should be conducted in a room free of outside odors. The observer should be cautioned to refrain from smoking or eating for an appreciable time before taking test. Odors should be washed from the hands prior to taking test.

3 The test should not be prolonged to a point where the sense of smell becomes fatigued.

IV PROCEDURE

A To obtain the approximate range of odor value take 50 ml, 14 ml and 5 ml of sample and make each sample up to 200 ml with odor-free water. Compare the odor of these three with 200 ml of odor-free water.

1 Cold odor: Bring dilutions to temperature of 24 - 25°C.

2 Shake each flask uniformly before smelling for odor. Observer should characterize type of odor.

3 Note which flasks contain odor and which do not. According to results obtained, prepare intermediate dilutions, in each case using sufficient odor-free water to make a total volume of 200 ml.

4 Include a flask with 200 ml of odor-free water with each series, as a blank for comparison.

B Arrange flasks so that their identity is unknown and bring to desired temperature.

1 Observe for odor and make chart with a "plus" or "zero" for each dilution.

2 The results are reported in "threshold odor numbers". The threshold odor number is calculated from the amount of sample in the most diluted portion which gives perceptible odor. The volume of the dilution (200 ml) divided by the volume of the sample in the dilution equals the threshold odor number. For example, if 5 ml diluted to 200 ml is the most dilute portion giving perceptible odor:

$$\frac{200}{5} = 40,$$ the threshold odor is numbered 40.

C The threshold odor number shall not be confused with the "threshold odor concentration". The threshold odor concentration is the smallest amount of odor-producing material in mg/1 required to give perceptible odor. If the threshold odor concentration is known, that value multiplied by the threshold odor number will give the concentration of the odor-producing material in the sample.

This outline was prepared by E. L. Robinson, Research Aquatic Biologist, Fish Toxicology Laboratory, 3411 Church Street, Newtown, OH 45244.

ROBERT A. TAFT SANITARY ENGINEERING CENTER
AQUATIC BIOLOGY

ALGAL THRESHOLD ODOR EXPERIMENT

Amount of Culture ______________ ml

Exp. No. ______________

Age of Culture ______________ days

Temp. Tested at ________ °C

No. Cells per ml ______________

Culture Medium ________

Mixed, Unialgal, Pure

Date ______________

Recorder ______________

Observer No.			1	2	3	4	5	6	7
Observer									
Flask No.	Culture No.	Dilution No.	R	R	R	R	R	R	R
Threshold Odor No.									
Description of Odor									

+ = Odor Detected   O = No Odor Detected

Remarks

Estimated Composite* T.O. No. ______________________

---

*Geometric average of T.O. No. of individual observers   E.L.R. 1956

DETERMINATION OF PLANKTON PRODUCTIVITY

I INTRODUCTION

Primary production is the synthesis of organic matter from inorganic raw materials. The energy required for this process may come from light (photosynthesis), or from chemical sources (chemosynthesis). The primary synthesis of organic matter in lakes and streams is carried on by planktonic and benthic algae and bacteria, and aquatic macrophytes.

II PHOTOSYNTHESIS

The photosynthetic process involves the uptake of CO_2 and the release of O_2. The reactions are enzyme catalyzed and are affected by the following factors:

A Temperature

B Light Intensity

C Light Quality

D pH

E Nutrients

F Trace Elements

III MEASURING PRODUCTIVITY

Methods employed to measure plankton productivity are:

A Standing Crop

B Oxygen

C pH

D Carbon-14

IV STANDING CROP METHOD

The productivity of a body of water is indicated, in a general way, by the density of the plankton population. The standing crop of plankton is commonly measured by determining one or more of the following:

A Dry and Ash-free Weight of Seston

B Cell or Unit Counts

C Cell Volume

D Chlorophyll

E Particulate and Dissolved Carbohydrate

F Particulate and Dissolved Organic Carbon

Increases in the standing crop over a period of time may be used to determine productivity. However, this method provides only a rough approximation of the rate of primary production.

V OXYGEN METHOD

The use of dissolved oxygen to determine short-term rates of primary production was introduced by Gaardner and Gran (1927). Estimates of the amount of carbon fixed are based on the premise that one molecule of oxygen is given off for each atom of carbon assimilated.

$$CO_2 + H_2O \rightarrow CH_2O + O_2$$

A "Light" and "dark" bottles are filled with sample and resuspended at various depths for 4 - 24 hours.

B The concentration of dissolved oxygen is determined (using the Winkler Method) at the beginning and end of the incubation period. The values obtained are as follows:

1 Final "light" bottle O_2 - initial O_2 = net photosynthesis

2 Initial O_2 - Final "dark" bottle O_2 = respiration

3 Net photosynthesis + respiration = gross photosynthesis

This method has some serious disadvantages:

A The bottles provide an artificial substrate for the proliferation of bacteria which use up large amounts of O_2, resulting in erroneously high respiration and low net photosynthesis values.

B The lower limit of sensitivity of the Winkler Method is 0.02 mg O_2/liter. This is a serious handicap when working in oligotropic lakes and the open sea.

VI CARBON-14 METHOD

The use of carbon-14 for the measurement of the rate of carbon assimilation by phytoplankton was pioneered by E. Steemann Nielsen (1952). The method is simple and very sensitive.

A Carbon-14 labelled sodium bicarbonate (4 - 10μc/liter) is added to "light" and "dark" bottles, which are resuspended in the water for 4 - 24 hours.

B An aliquot of the sample is passed through a membrane filter (1.2 μ pore diameter), and the filters are treated with acid to remove any inorganic labelled carbon.

C The (beta) activity of the filter is determined with an end-window Geiger tube, or with gas flow or liquid scintillation techniques.

D The carbon fixed is determined as follows:

$$\text{carbon fixed} = \frac{\text{activity on filter}}{\text{total activity added}} \times \text{available } HCO_3^- \times \begin{matrix}\text{correction for}\\ \text{isotope}\\ \text{discrimination}\end{matrix}$$

There are several important disadvantages in this method.

A Some of the labelled photosynthesis products will be broken down immediately by respiration, and the liberated carbon-14 reused in photosynthesis. Therefore, it is generally agreed that the method measures only net photosynthesis.

B It has been found that the algae rapidly excrete up to 50% of the photosynthate in the form of organic acids, carbohydrates, and amino acids. Since these labelled materials are not retained by the filter, they escape detection.

VII pH METHOD

The uptake of CO_2 by the algae during photosyntheses results in an increase in the pH of the surrounding medium. Periodic pH measurements are made of the body of water being studied, and the carbon uptake is determined using published nomographs.

Verduin (1952) used this method in a study of the productivity of Lake Erie. However, the method has not gained wide acceptance because it can be used only in waters with low alkalinity.

REFERENCES

1 Allen, M.B. Excretion of Organic Compounds by Chlamydomonas. Arch. f. Mikrobiol. 24:163-168. 1956.

2 Curl, H. Jr., and Small, L.F. Variations in Photosynthetic Assimilation in Natural Marine Photoplankton Communities. Limnol. Oceanogr. 10(Suppl.):R67-R73. 1965.

3 Gaardner, T., and Gran, H.H. Investigations of the Production of the Plankton in the Oslo Fjord. Rapp. et Proc. - Verb., Con. Internat. Explor. Mer. 42:1-48. 1927.

4 Goldman, C. R. Molybdenum as a Factor Limiting Primary Productivity in Castle Lake, California. Science 132: 1016-1017. 1960.

5 Kamen, M. D. Primary Processes in Photosynthesis. Academic Press, New York. 1963.

6 Marshall, S. M., and Orr, A. P. Carbohydrate as a Measure of Photoplankton. J. Mar. Biol. Assoc. U. K. 42:511-519. 1962.

7 Ryther, J. H. Photosynthesis in the Ocean as a Function of Light Intensity. Limnol. Oceanogr. 1:61-70. 1956.

8 Steemann Nielsen, E. The Use of Radioactive Carbon (C-14) for Measuring Organic Production in the Sea. J. Con. Internat. Explor. Mer. 18:117-140. 1952.

9 Strickland, J. D. H. Measuring the Production of Marine Phytoplankton. Bull. Fish. Res. Bd. Can. No. 122: 1-172. 1960.

10 Verduin, J. Photosynthesis and Growth Rates of Two Diatom Communities in Western Lake Erie. Ecology 33(2): 163-168. 1952.

11 Vernon, L. P. Bacterial Phytosynthesis. Ann. Rev. Plant. Physiol. 15:73-100. 1962

12 Wetzel, R. G. A Comparative Study of the Primary Productivity of Higher Aquatic Plants, Periphyton, and Phytoplankton in a Large, Shallow Lake. Internat. Rev. Hydrobiol. 49:1-61. 1964.

13 Yentsch, Charles S. The Measurement of Chloroplastic Pigments- Thirty Years of Progress? pp. 255-270 in Chemical Environment in the Aquatic Habitat. Proc. IBP Symposium. Amsterdam. 1967. (N.V. Noord-Hollandsche Uitgevers Maatschappij. Amsterdam, Netherlands. 8.95)

This outline was prepared by C. I. Weber, Chief, Biological Methods Branch, Analytical Quality Control Laboratory, NERC, EPA, Cincinnati, OH 45268.

LABORATORY: PROPORTIONAL COUNTING OF PLANKTON

I OBJECTIVE

To learn and practice the techniques of proportional counting of mixed plankton samples.

II MATERIALS

A Several plankton samples, each containing a number of plankton forms.

B Class slides, cover slips, and dropping pipets.

III PROCEDURES

A Make an ordinary wet mount of the sample provided.

B Scan the slide. Identify and list all types of plankton present.

C Proportional Counting (use clump count)

1 Field count

a Count and tally all individuals of each type present in a field. The best way to do this is to list the most common types separately and record the counts and then enumerate the other forms.

b Move the slide at random and repeat the process. Do this for 5 or 10 fields, or for one or two strips.

c Tally the results and compute the percent of each type.

2 Five hundred count

a Moving the slide at random count and tally all the types of plankton as before until a total of 500 cells or clumps have been counted.

b Tally the results and compute the percentage of each type as before.

IV RESULTS

A Record your results for both methods on the board.

B Discuss the two methods and the use of the proportional count results.

This outline was prepared by M. E. Bender, Biologist, formerly with FWPCA Training Activities, SEC.

LABORATORY: CALIBRATION OF PLANKTON COUNTING EQUIPMENT

I OBJECTIVES

A To Become Familiar with Microscope Calibration Procedures

B To Calibrate the Particular Equipment Assigned to you

II MATERIALS

A Whipple, Plankton Counting Reticule

B Compound Microscope as Assigned

C Stage Micrometer

III PROCEDURE

A Adjust the interpupillary distance to the position most comfortable for your eyes, and record the setting on the "Microscope Calibration Data" sheet.

B Install a Whipple plankton counting reticule in the right eyepiece.

C Obtain a stage micrometer and focus on the scale at 100X magnification.

D Record the exact dimensions of the entire field in the column marked "Whole" on the plate "Microscope Calibration Data."

E Do the same with the 200X and 400X magnifications.

F Return the stage micrometer to the supply table.

G Values for the "Large" and "Small" columns may now be calculated arithmetically. There are ten large squares across the whole field, and 5 small squares across the large square which is subdivided, in the center of the field.

H Calculate the conversion factors to counts per ml according to the formulae in the lecture entitled "Calibration and Use of Plankton Counting Equipment."

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, DTTB, OWP, EPA, Cincinnati, OH 45268.

MICROSCOPE CALIBRATION DATA

Microscope No. ______________

Approximate Magnification	Tube Length, or Interpupillary Setting	Linear dimensions of Whipple squares in millimeters*			Factor for Conversion to count/ml
		Whole	Large	Small	
100X, obtained with					**(2 S-R Strips)**
Objective Serial No.					
________					
and Ocular Serial No.					
________					
200X, obtained with					**(2 S-R Strips)**
Objective Serial No.					
________					
and Ocular Serial No.					
________					
400X, obtained with					**(Nannoplankton)** **(cell-20 fields)**
Objective Serial No.					
________					
and Ocular Serial No.					
________					

*1mm = 1000 microns

BI. AQ. pl. 8. 10. 60.

MICROSCOPE CALIBRATION DATA

Microscope No. ______________

Approximate Magnification	Tube Length, or Interpupillary Setting	Linear dimensions of Whipple squares in millimeters*			Factor for Conversion to count/ml
		Whole	Large	Small	
100X, obtained with					**(2 S-R Strips)**
Objective Serial No.					
______					
and Ocular Serial No.					
______					
200X, obtained with					**(2 S-R Strips)**
Objective Serial No.					
______					
and Ocular Serial No.					
______					
400X, obtained with					**(Nannoplankton) (cell-20 fields)**
Objective Serial No.					
______					
and Ocular Serial No.					
______					

---

*1mm = 1000 microns

BI. AQ. pl. 8. 10. 60.

LABORATORY: FUNDAMENTALS OF QUANTITATIVE COUNTING

I OBJECTIVE

To learn and practice the basic techniques of quantitative plankton counting

II MATERIALS

A Plankton Samples Containing a Variety of Plankton Forms

B S-R Cells and Coverglasses, Large Bore 1 ml Pipettes, Whipple Discs, Plankton Record Form

III PROCEDURE

A Fill the S-R cell with sample number 1 as follows:

> Place the coverglass diagonally across the S-R cell. This leaves the other two corners uncovered; one for putting in the sample fluid, the other to allow air to be driven out as it is replaced by the incoming aliquot. Shake the sample to disperse the plankton. Before settling occurs in the sample draw about 1-1/4 ml of the fluid into the pipette and quickly fill the S-R cell by delivering the aliquot into one of the open corners of the chamber.

B Using 100x focus on the sample. After focus has been obtained switch to 200x. Scan the slide and list the plankton forms present.

C Starting from one end of the S-R cell and proceding to the opposite (this is called a strip count, begin counting (clump counts) the plankton forms. The length of the cell may be traversed in several ways.

1 Count all the forms in the Whipple square or in a portion of the square, record the count and move the slide so that the square covers the adjoining area.

2 Move the slide very slowly counting and recording the various forms as they pass the leading edge of the Whipple disc.

IV RESULTS

A Using the conversion factor obtained in the previous laboratory compute the number of plankton organisms per ml.

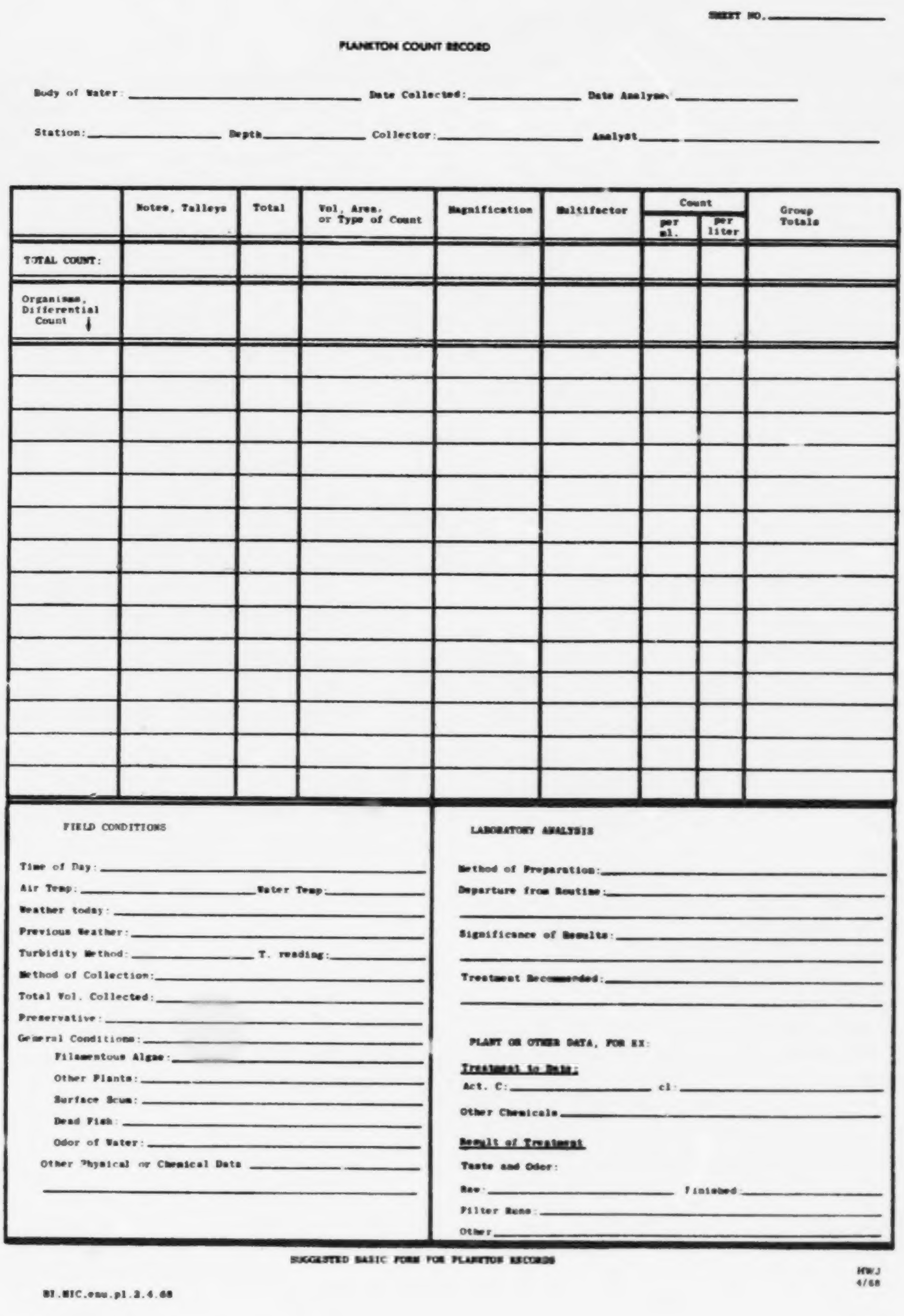
B Record the results on the board.

C Discussion of Results

D Refill the slide with a fresh aliquot and recount the sample. Compare results with the first count.

E Count the other samples of mixed plankton as assigned, following the same procedure.

This outline was prepared by M. E. Bender, Former Biologist, FWPCA, Water Pollution Training Activities, SEC.



SHEET NO. ____________

PLANKTON COUNT RECORD

Body of Water: ______________ Date Collected: ____________ Date Analyzed: ____________

Station: ____________ Depth ____________ Collector: ____________ Analyst ____________

	Notes, Talleys	Total	Vol, Area, or Type of Count	Magnification	Multifactor	Count		Group Totals
						per ml.	per liter	
TOTAL COUNT:								
Organisms, Differential Count ↓								

FIELD CONDITIONS

Time of Day: ____________
Air Temp: ____________ Water Temp: ____________
Weather today: ____________
Previous Weather: ____________
Turbidity Method: ____________ T. reading: ____________
Method of Collection: ____________
Total Vol. Collected: ____________
Preservative: ____________
General Conditions: ____________
Filamentous Algae: ____________
Other Plants: ____________
Surface Scum: ____________
Dead Fish: ____________
Odor of Water: ____________
Other Physical or Chemical Data ____________

LABORATORY ANALYSIS

Method of Preparation: ____________
Departure from Routine: ____________
Significance of Results: ____________
Treatment Recommended: ____________

PLANT OR OTHER DATA, FOR EX:

Treatment to Date:
Act. C: ____________ cl: ____________
Other Chemicals ____________

Result of Treatment

Taste and Odor:
Raw: ____________ Finished: ____________
Filter Runs: ____________
Other ____________

SUGGESTED BASIC FORM FOR PLANKTON RECORDS

HWJ
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ALGAL GROWTH POTENTIAL TEST

I INTRODUCTION

Dense growths of algae in surface waters are aesthetically undesirable, cause problems in water treatment, produce changes in the aquatic environment that are harmful to fish and other aquatic life, and are symptomatic of pollution. The density of phytoplankton populations is directly related to the concentration of nutrients. This relationship has been well documented, and is now embodied in the concept of *trophic level* or *trophic status* of surface waters. One or more of the following parameters are commonly used to describe the trophic status: (a) nutrient concentration - principally N and P, (b) algal count, (c) chlorophyll concentration, (d) primary productivity, (e) particulate organic matter, (f) oxygen depletion in the hypolimnion, and (g) phytoplankton species composition or indicator species (Rawson 1956; Davis 1964; Goldman & Carter 1965; Oglesby & Edmondson 1966; Fruh, Steward, Lee & Rohlich 1966).

II EUTROPHICATION

Three general trophic levels now recognized, here arranged in ascending order, are: oligotrophic (low), mesotrophic (intermediate), and eutrophic (high). The addition of nutrients to surface waters raises the trophic level and results in an increase in phytoplankton density and changes in the species composition. This process, commonly referred to as eutrophication, is greatly accelerated by the discharge of nutrient-laden domestic and industrial wastes (Hasler 1947), Edmondson & Anderson 1956).

III MEASUREMENTS OF TROPHIC LEVELS

Although chemical analyses provide information on the concentration of nutrients, their availability to the algae can be determined only by biological assay. Biological assays to determine the potential (algal) productivity of surface water were first used in the late twenties (Schreiber 1927) and early thirties (Strom 1933), but until recently had been used only infrequently (Potash 1956; Skulberg 1964, 1967; Shelef & Halperin 1970). In 1967, the Joint Industry-Government Task Force on Eutrophication took steps to develop a standardized algal growth potential (AGP) test. Using this test, one can:

A Evaluate the effectiveness of waste treatment processes in removing elements that support or stimulate the growth of algae.

B Determine at what point along the time scale of progressing eutrophication the water of a given lake or stream happens to lie (trophic status).

C Anticipate the effect on algal production of introducing extraneous nutrients.

D Determine the extent to which nutrient levels must be reduced in a body of water to effect an acceptable remedy.

IV BASIC STEPS OF ALGAL GROWTH POTENTIAL TEST

A A surface (test) water sample is collected and the indigenous microorganisms are removed by filtration (0.45 micron membrane filter at 15 inches of mercury) or ultracentrifugation.

B The surface water and standard medium (Table 1) are inoculated with 1000 cells/ml of *Selenastrum capricornutum*, or 50,000 cells/ml of *Anabaena flos-aquae* or *Microcystis aeruginosa*.

C The cultures are prepared in triplicate and incubated 7-10 days at 24°C, 200 fc (blue-greens) or 400 fc (*Selenastrum*) continuous illumination, with shaking at 100 oscillations/min (culturing may be by flask, chemostat, or *in situ* technique).

D Algal growth is measured daily by (1) cell counts, (2) determining the

TABLE 1

MAY, 1970 VERSION OF PAAP NUTRIENT BASAL MEDIUM

(This formula consists of 30% of the concentrations of the macroelements listed in the February, 1969, PAAP Booklet. The Na_2CO_3 was replaced by $NaHCO_3$, and the EDTA was reduced to 333 μg/l.)

MACROELEMENTS: (milligrams per liter)

Compound	Final Conc.	Element Furnished	Element Conc.
$NaNO_3$	25.500	N	4.200
K_2HPO_4	1.044	P	0.186
		K	0.469
$MgCl_2$	5.700	Mg	1.456
$MgSO_4 \cdot 7H_2O$	14.700	Mg	1.450
		S	1.911
$CaCl_2 \cdot 2H_2O$	4.410	Ca	1.202
$NaHCO_3$	15.000	Na	11.001

If the medium is to be filtered, add the following trace-element-iron-EDTA solution from a single combination stock solution after filtration. With no filtration, K_2HPO_4 should be added last to avoid iron precipitation. Stock solutions of individual salts may be made up in 1000 ×'s final conc. or less.

MICROELEMENTS: (micrograms per liter)

H_3BO_3	185.5	B	32.5
$MnCl_2$	264.3	Mn	115.4
$ZnCl_2$	32.7	Zn	15.7
$CoCl_2$	0.780	Co	0.354
$CuCl_2$	0.009	Cu	0.004
$Na_2MoO_4 \cdot 2H_2O$	7.26	Mo	2.88
$FeCl_3$	96.0	Fe	33.05
$Na_2EDTA \cdot 2H_2O$	300.0		

chlorophyll content, in vivo fluorescence, light scattering or optical density (600 nm) of the culture, (3) measuring the C-14 uptake, or (4) determining the dry weight of the algae at the end of the incubation period. Regardless of the parameter used to measure growth response, the result should always be expressed in terms of the final dry weight of the culture.

E The growth response of the alga in the test water is compared to its growth in the standard medium.

V PHASES OF THE TEST STILL UNDER STUDY INCLUDE:

A Composition of the standard growth medium.

B Effects of ventilation and shaking on the growth response of batch cultures.

C Techniques of measuring growth response.

D Techniques of removing indigenous microorganisms from test surface waters.

VI For copies of the Provisional Algal Assay Procedure and information on the availability of subcultures of the test organism, contact:

Dr. A. F. Bartsch, Chairman
JTF Research Program Group
Director, Pacific Northwest
Water Research Laboratory
Corvallis, Oregon 97330

REFERENCES

1 _________. Provisional Algal Assay Procedure. Joint Industry-Government Task Force on Eutrophication, P.O. Box 3011, Grand Central Station, N.Y. 10017. 1969.

2 Davis, C.C. Evidence for the eutrophication of Lake Erie from phytoplankton records. Limnol. Oceanogr. 9:275. 1964.

3 Edmondson, W.T. and Anderson, G.C. Artificial eutrophication of Lake Washington. Limnol. Oceanogr. 1 (1):47-53. 1956.

4 Fruh, E.G., Stewart, K.M., Lee, G.F., and Rohlich, G.A. Measurements of Eutrophication and Trends. JWPCF 38(8):1237-1258. 1966.

5 Goldman, C.R. and Carter, R.C. An investigation by rapid C^{14} bioassay of factors affecting the cultural eutrophication of Lake Tahoe, California. JWPCF 37:1044-1063. 1965.

6 Hasler, A.D. Eutrophication of lakes by domestic drainage. Ecology 28(4): 383-395. 1947.

7 Oglesby, R.T. and Edmondson, W.T. Control of Eutrophication. JWPCF 38(9):1452-1460. 1966.

8 Potash, M. A biological test for determining the potential productivity of water. Ecology 37(4):631-639. 1956.

9 Rawson, D.S. Algal indicators of lake types. Limnol. Oceanogr. 1:18-25. 1956.

10 Schreiber, W. Der Reinkultur von marinem Phytoplankton und deren Bedeutung fur die Erforschung der Produktions-fahigkeit des Meerwassers. Wissensch. Meeresunters., N.F. 16:1-34. 1927.

11 Shelef, G. and Halperin, R. 1970. Wastewater nutrients and algae growth potential. In: H.I. Shuval, ed., "Developments in Water Quality Research", Proc. Jerusalem Internat'l. Conf. on Water Quality and Poll. Res., June, 1969. Ann Arbor-Humphrey Science Publ., p. 211-228.

12 Skulberg, O.M. Algal problems related to the eutrophication of European water supplies, and a bioassay method to assess fertilizing influences of pollution on inland waters. In: D.F. Jackson, ed., "Algae and Man", Plenum Press, N. Y. p. 262-299. 1964.

13 Skulberg, O.M. Algal cultures as a means to assess the fertilizing incluence of pollution. In: Advances in Water Pollution Research, Volumn 1, Pergamon Press, Washington, D. C. 1967.

14 Strom, K.M. Nutrition of algae. Experiments upon; the feasibility of the Schreiber method in fresh waters; the relative inportance of iron and manganese in the nutritive medium; the nutritive substance given off by lake bottom muds. Arch. Hydrobiol. 25:38-47. 1933.

ADDITIONAL RECENT REPORTS:

1 ______________. Algal Assay Procedure Bottle Test. 82 pp. Environmental Protection Agency, National Eutrophication Research Program, Corvallis, Oregon. 1971.

2 ______________. Inter-Laboratory Precision Test. An Eight-Laboratory Evaluation of the Provisional Algal Assay Procedure Bottle Test. 70 pp. Environmental Protection Agency, National Eutrophication Research Program, Corvallis, Oregon. 1971.

3 Berge, G., Predicted Effects of Fertilizers Upon the Algae Production in Fern Lake. Fisk Dir. Skr. Serv. Hav. Unders., 15:339-355. 1969.

4 Johnson, J.M., T.O. Odlaug, T.A. Olson, and O.R. Ruschmeyer. The Potential Productivity of Freshwater Environments as Determined by an Algal Bioassay Technique. Water Resources Research Center Bulletin No. 20, University of Minnesota, Minneapolis. 1970.

5 Maloney, T.E., W.E. Miller, and T. Shiroyama. Algal Responses to Nutrient Additions in Natural Waters. I. Laboratory Assays. In: Special Symposia I:134-140. Amer. Soc. Limnol. Oceanogr. 1972.

6 Miller, W.E., and T.E. Maloney. Effects of Secondary and Tertiary Wastewater Effluents on Algal Growth in a Lake-River System. JWPCF 43(12)2361-2365. 1971.

7 Murray, S., J. Scherfig, and P.S. Dixon. Evaluation of Algal Assay Procedures-PAAP Batch Test. JWPCF 43(10): 1991-2003. 1971.

8 Shapiro, J., and R. Riberiro. Algal Growth and Sewage Effluent in the Potomac Estuary. JWPCF 37(7): 1034-1043. 1965.

9 Toerien, D.F., C.H. Huang, J. Radimsky, E.A. Pearson, and J. Scherfig. Final Report, Provisional Algal Assay Procedures. 211 pp. Sanitary Engineering Research Laboratory Report No. 71-6, University of California, Berkeley. 1971.

This outline has been prepared by Dr. C. I. Weber, Chief, Biological Methods Section, Analytical Quality Control Laboratory, NERC, EPA, Cincinnati, OH 45268.

ALGAE AND ACTINOMYCETES IN WATER SUPPLIES

I Water treatment always should include detection and control of microorganisms.

A Two types of microorganisms are involved:

1 Pathogenic types include such forms as the typhoid bacteria, the dysentery ameba, and the infectious hepatitis virus.

2 Interference types include taste and odor organisms, filter-clogging organisms, pipe-infesting organisms, and others.

B Water treatment practices are closely associated with these organisms.

1 For pathogens, practices include coliform tests, use of chlorine, and guarding the water supply against fecal pollution.

2 For interference organisms, practices include plankton enumeration, use of copper sulfate and the covering of reserviors.

3 Many of the other treatment practices have significant effects on the organisms.

C This discussion will be limited to the interference organisms.

II EXAMPLES OF PROBLEMS CAUSED BY INTERFERENCE ORGANISMS

A At Chicago, the alga Dinobryon reappears almost every year, generally in June and July in numbers sufficient to impart a prominent fishy odor to the water. In 1951, it required an estimated $70,500 worth of activated carbon to control the odor of this organism for a period of two months.

B At Indianapolis, copepods were present in parts of the distribution system in numbers sufficient to be visible in the drinking water. The eggs of the copepods were found to pass through the filters and to hatch in the distribution system.

C At Oklahoma City, prominent earthy odors have appeared frequently. The organisms blamed for this trouble are the mold-like actinomycetes.

D At Peoria, white wigglers up to 3/8" long were reported in the tap water, during early March, 1956. These chironomid larvae had hatched in the city's open reservoir, requiring that the reservoir be drained, cleaned and treated with a larvicide.

E At Chicago, diatoms are a very important cause of short filter runs. The one diatom Tabellaria is considered to be more responsible than any other organism for this trouble.

F In Ontario, the alga Cladophora often grows in large numbers attached to rocks on the shoreline of lakes. When the alga is broken loose it collects near the shoreline and gives rise to very offensive odors.

G In a water supply impoundment in Utah the plankton algae frequently cause the pH of the water to increase to 8.3 or higher, requiring that the water be treated with acid to obtain the desired pH of 8 or lower.

H In Texas a water supply from underground sources was stored in a large open settling basin. Oscillatoria and unicellular green algae developed in large numbers in the stored water, turning it green and producing a strong odor.

I Los Angeles has more than 25 open reservoirs of various sizes and ranging in elevation from almost sea level to over

7,000 feet. Many tons of copper sulfate are used every year in these reservoirs for rigid control of plankton, chiefly diatoms and occassionally blue-green algae. This treatment is carried out to improve the water quality including the reduction of tastes and odors.

III TYPES OF PROBLEMS CAUSED BY INTERFERENCE ORGANISMS

A Tastes and Odors

1 May be caused by algae, actinomycetes, crustacea, and anaerobic bacteria.

2 Common algal odors imparted to water are ones described as fish, earthy, musty, grassy, cucumber, geranium, nasturtium, and septic.

3 Common actinomycete odor is earthy.

4 Tastes produced in water by algae include sweet and bitter.

5 Other causative agents of tastes and odors may be industrial wastes, sludge, and compounds dissolved from soil and rock, and chemicals used in treatment.

B Filter Clogging

1 Both rapid and slow sand filters are affected.

2 Diatoms are the organisms most frequently involved but blue-green algae, filamentous green algae and other organisms as well as silt may cause it.

C Other Problems in the Treatment Plant

1 Algae may cause variation in the pH, hardness, color, and organic content of the water.

2 Amount of plankton organisms often influences the rate and effectiveness of coagulation.

3 Chlorine dosage may depend upon amount of plankton organisms present.

4 Growths of algae may reduce the flow through influent channels and screens.

5 Organisms may be responsible for increasing the quantity of sludge to be disposed of in sedimentation basins.

6 Microcrustacea "spot" paper in paper mill rolls.

D Infestation of Distribution Systems

1 Attached organisms reduce the rate of flow in the pipes.

2 Iron and sulfur bacteria may initiate or stimulate corrosion of pipes.

3 Organisms may appear as visible bodies in tap water.

4 Tastes and odors may result from presence of organisms.

5 Chlorine residual is difficult to maintain when organic matter is present.

6 Organisms could theoretically harbor and protect against chlorine certain pathogenic bacteria.

E Profuse Growths of Organisms in Raw Water Supplies

1 A limited and balanced growth of various organisms is generally an asset.

2 Extensive surface mats, blooms and marginal growths often cause troubles along the shoreline and eventually in the treatment plant.

3 Some fish kills may be caused by profuse growths of algae by reducing the DO during the night.

4 Certain massive growths of blue-green algae are deadly poisonous to animals.

IV ORGANISMS INVOLVED

A Animal forms include protozoa, rotifers, crustaceans, worms, bryozoans, fresh water sponges, water mites and larval stages of various insects.

B Plant forms include algae, actinomycetes and other bacteria, molds and larger aquatic green plants.

V IMPORTANCE OF BIOLOGICAL PROBLEMS

A The increased use of surface water supplies increases the problems caused by organisms. Biological problems are less common with ground water supplies.

B Standards of water quality requested by domestic and industrial patrons are rising.

C Procedures for detection, control and prevention of problems caused by organisms are improving and are receiving more extensive use.

VI A number of methods may be used to control the interference organisms or their products:

A Addition to water or an algicide or pesticide such as copper sulfate, chlorine dioxide or copper-chlorine-ammonia.

B Mechanical cleaning of distribution lines, settling basins, sand filters, screens, and reservoir walls.

C Modification of coagulation, filtration, chemical treatment, or location of intake.

D Use of absorbent, such as activated carbon, for taste and odor substances.

E Modification of Reservoir to Reduce the Opportunities for Massive Growths of Algae

1 By covering treated water reservoir to exclude sunlight

2 By increasing the depth of the water in reservoirs

3 By eliminating shallow marginal areas

4 By reducing the amount of fertilizing nutrients entering the reservoir.

VII It is generally more satisfactory to anticipate and prevent problems due to these organisms than it is to cope with them later.

A Routine biological tests are essential to detect the initial development or presence of interference organisms.

1 Control measures can then be used before problem becomes acute.

2 These tests should be applied to the raw treatment plant water supply and distribution system.

B In the Reservoir or Other Raw Water Supply

1 Routine plankton counts should be made of water samples from selected locations. Plankton counter should be aware of the particular organisms known to be most troublesome.

2 During the warmer months routine surveys of the reservoir, lake or stream should be made to record any visible growths of algae and other organisms.

3 Odor tests of water from several locations should be made to obtain advance notice of potential trouble at the treatment plant.

C In the Treatment Plant

1 Records of plankton counts and threshold odor between each step in treatment gives data on effectiveness of each procedure.

2 Coagulation and filtration can be adjusted to remove up to 95% or more of organisms in water.

3 Microscopic analysis of samples of filter material for organisms can supply data useful in modifying sand filtration and treatment of finished water.

D In the Distribution System With Its Finished Water

1 Open reservoirs require constant attention especially during summer.

2 Parts of the system farthest from the treatment plant or adjacent to dead ends require most frequent sampling for organisms and tastes and odors.

VIII SUMMARY

A Interference organisms cause problems in distribution systems, treatment plants, raw water supplies.

B Organisms involved include algae, actinomycetes, other bacteria, and minute aquatic animals.

C Control is by special chemicals, mechanical cleaning, adjustment of chemical or mechanical treatment and by modification of reservoirs, intakes, etc., for the raw water supply.

D Facilities for detection of problems in their early stages are required for most efficient and satisfactory control.

REFERENCES

1 Palmer, C. M. Algae in Water Supplies. An Illustrated Manual on the Identification, Significance, and Control of Algae in Water Supplies. U.S. Public Health Service Publication No. 657. 1959. p. 88.

2 Palmer, C.M. and Poston, H.W. Algae and Other Interference Organisms in Indiana Water Supplies. Jour. Amer. Water Works Assn. 48:1335-1346. 1956.

3 Palmer, C.M. Algae and Other Interference Organisms in New England Water Supplies. Jour. New England Water Works Assn. 72:27-46. 1958.

4 Palmer, C.M. Algae and Other Organisms in Waters of the Chesapeake Area. Jour. Amer. Water Works Assn. 50:938-950. 1958.

5 Palmer, C.M. Algae and Other Interference Organisms in the Waters of the South Central United States. Jour. Amer. Water Works Assn. 52:897-914. 1960.

6 Silvey, J.K. and Roach, A.W. Actinomycetes May Cause Tastes and Odors in Water Supplies. Public Works 87. 5:103-106, 210, 212. 1956.

7 Ingram, W.M. and Bartsch, A.F. Operators Identification Guide to Animals Associated with Potable Water Supplies. Jour. Amer. Water Works Assn. 52:1521-1550. 1960.

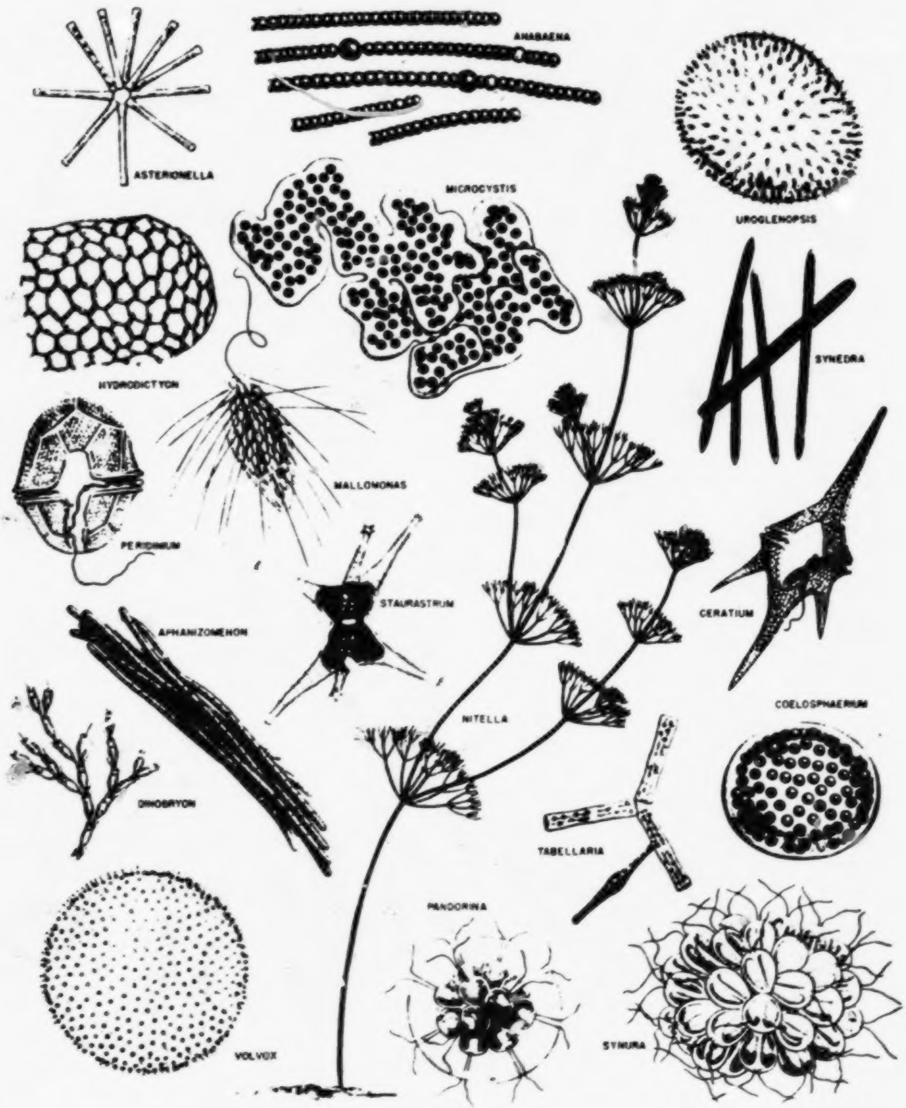
8 Otto, N.E. and Bartley, T.R. Aquatic Pests on Irrigation Systems. Identification Guide. Bur. of Reclamation. USDI. 72 pp. 1965.

9 Herbst, Richard P. Ecological Factors and the Distribution of Cladophera glomerata in the Great Lakes. Amer. Midl. Nat. 82:90-98. 1969.

This outline was prepared by C.M. Palmer, formerly Aquatic Biologist, In Charge, Interference Organism Studies, Microbiology Activities, Research & Development, Cincinnati Water Research Laboratory, FWPCA.

ALGAE IMPORTANT IN WATER SUPPLIES

TASTE AND ODOR ALGAE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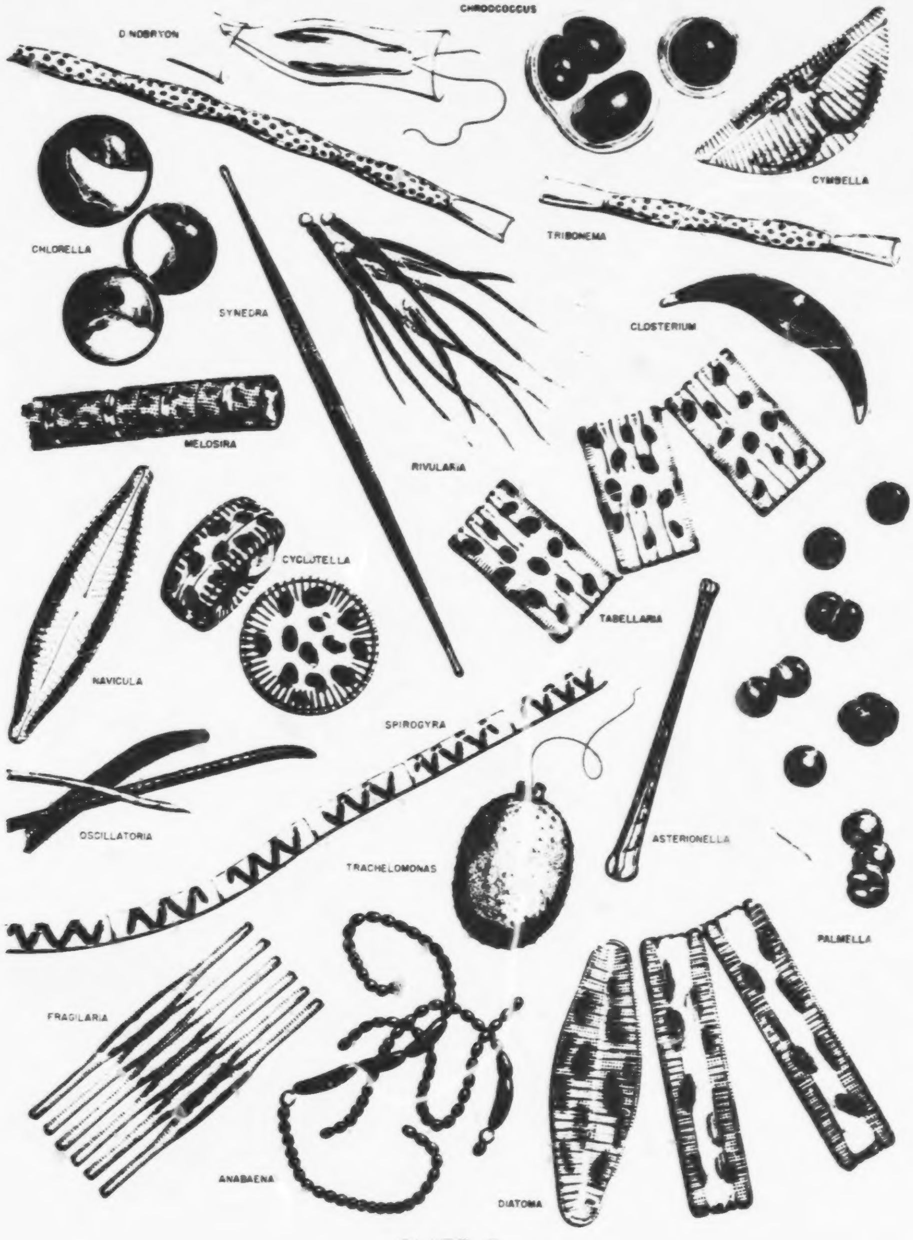
CERATIUM
APHANIZOMENON
NITELLA
COELOSPHAERIUM
DINOBRYON
TABELLARIA
PANDORINA
VOLVOX
SYNURA

PLATE I

FILTER CLOGGING ALGAE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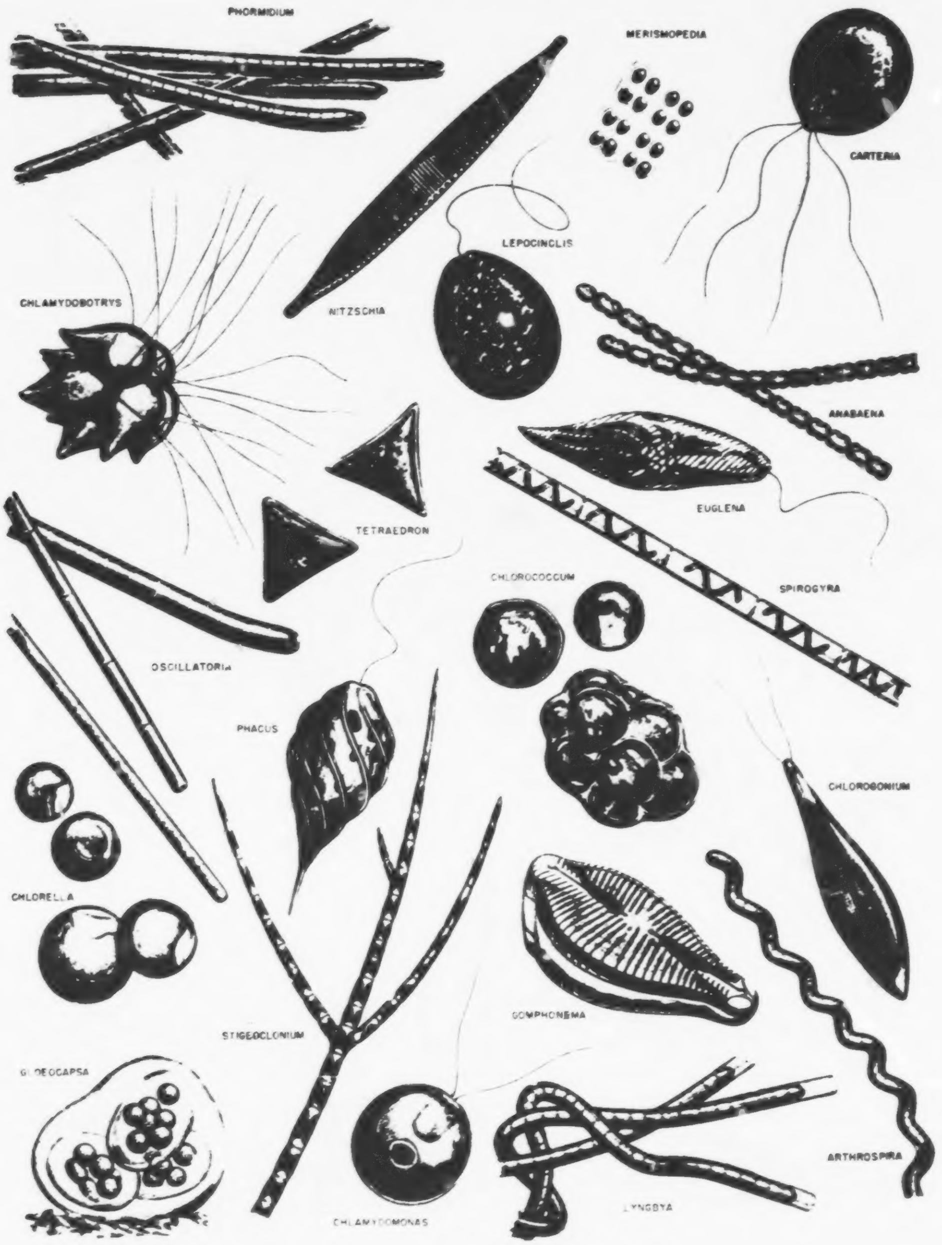
TABELLARIA
NAVICULA
SPIROGYRA
OSCILLATORIA
ASTERIONELLA
TRACHELOMONAS
PALMELLA
FRAGILARIA
ANABAENA
DIATOMA

PLATE 2

POLLUTED WATER ALGAE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NITZSCHIA
ANABAENA
EUGLENA
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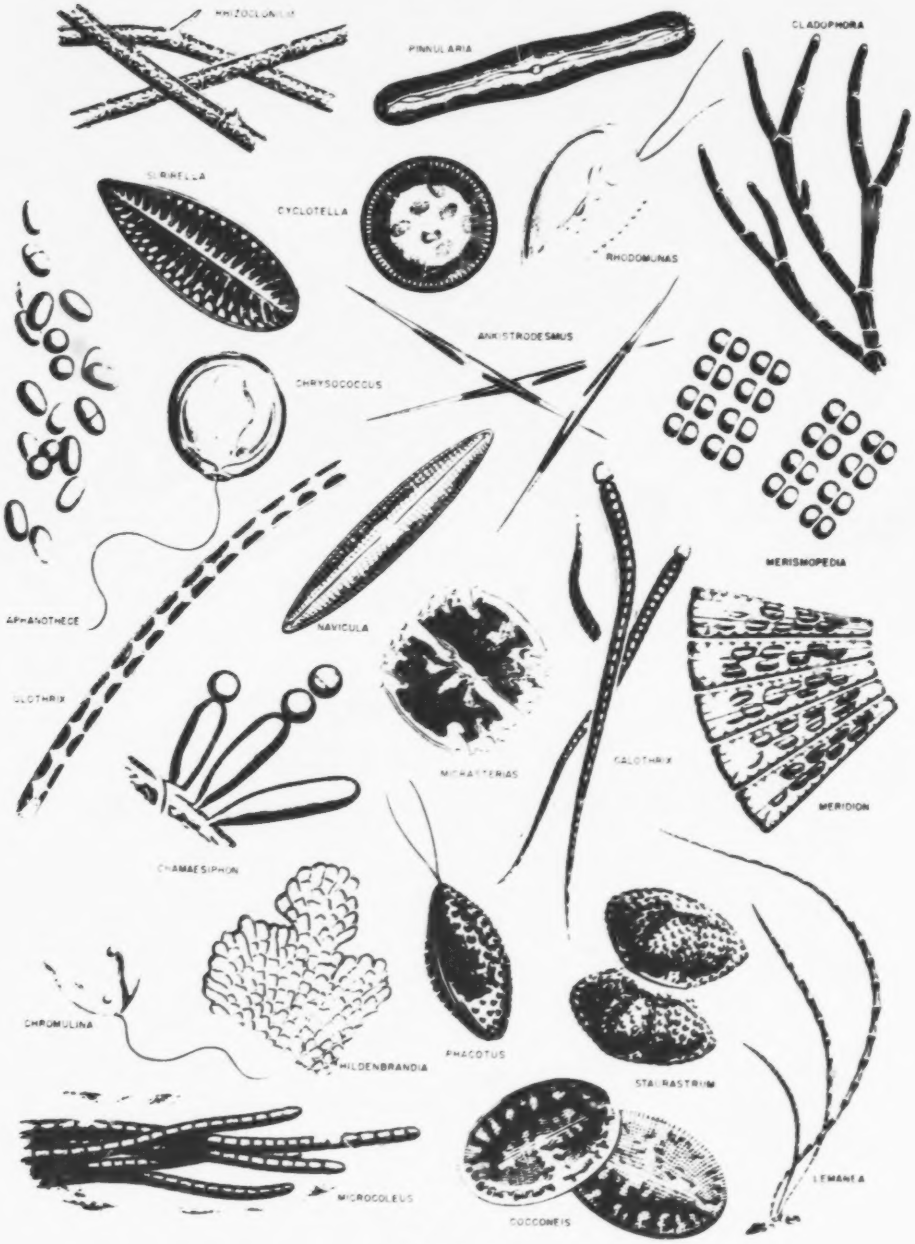
SPIROGYRA
CHLOROCOCCUM
OSCILLATORIA
PHACUS
CHLOROGONIUM
CHLORELLA
GOMPHONEMA
STIGEOCLONIUM
GLOEOCAPSA
ARTHROSPIRA
LYNGBYA
CHLAMYDOMONAS

PLATE 3

CLEAN WATER ALGAE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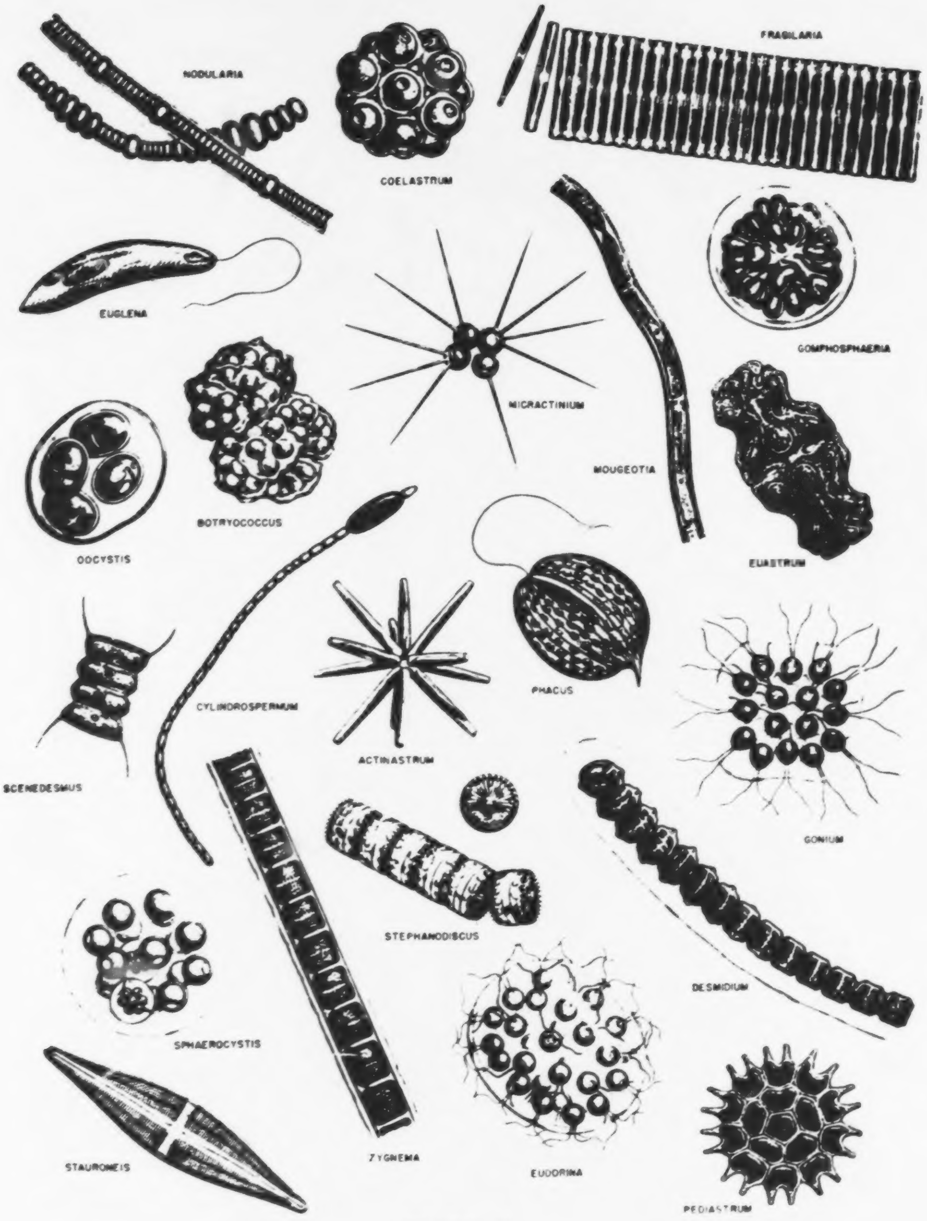
PINNULARIA
CLADOPHORA
SURIRELLA
CYCLOTELLA
RHODOMONAS
ANKISTRODESMUS
CHRYSOCOCCUS
MERISMOPEDIA
APHANOTHECE
NAVICULA
ULOTHRIX
MICRASTERIAS
CALOTHRIX
MERIDION
CHAMAESIPHON
CHROMULINA
HILDENBRANDIA
PHACOTUS
STAURASTRUM
LEMANEA
MICROCOLEUS
COCCONEIS

PLATE 4

SURFACE WATER ALGAE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MOUGEOTIA
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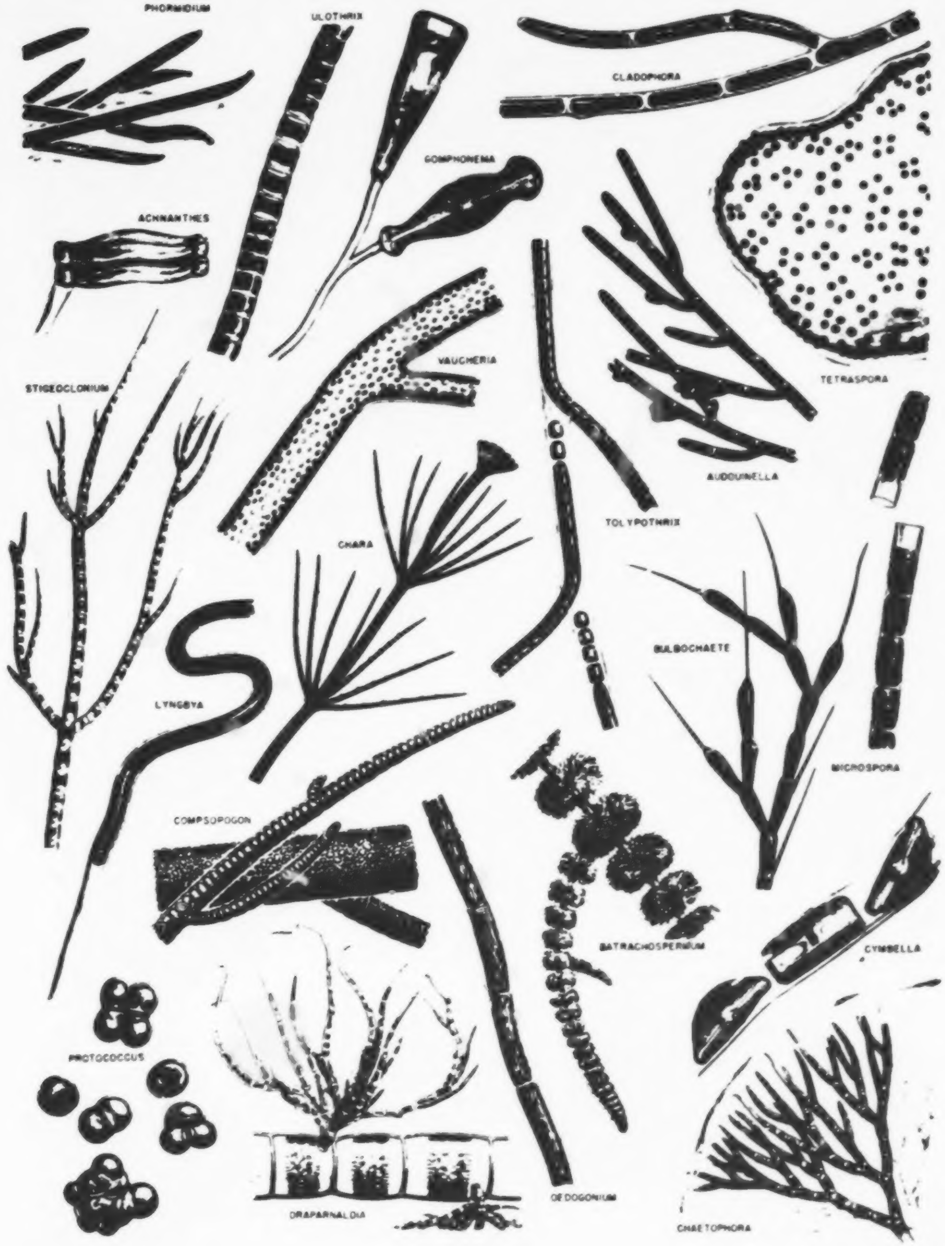
OOCYSTIS
EUASTRUM
CYLINDROSPERMUM
PHACUS
ACTINASTRUM
SCENEDESMUS
GONIUM
STEPHANODISCUS
DESMIDIUM
SPHAEROCYSTIS
STAURONEIS
ZYGNEMA
EUDORINA
PEDIASTRUM

PLATE 5

ALGAE GROWING ON RESERVOIR WALLS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CHARA
TOLYPOTHRIX
BULBOCHAETE
LYNGBYA
MICROSPORA
COMPSOPOGON
BATRACHOSPERMUM
CYMBELLA
PROTOCOCCUS
DRAPARNALDIA
OEDOGONIUM
CHAETOPHORA

PLATE 6

ALGAE AS INDICATORS OF POLLUTION

I LIMITATIONS

A Algae are only one of a number of types of organisms present which could be considered.

B Forms recognized here as algae are comparatively simple, pigmented, aquatic organisms, including blue-greens, greens, diatoms and pigmented flagellates.

C Various pollutants react differently on algae. Organic pollutants such as household sewage will be dealt with here.

D No algae are intestinal organisms. They therefore are not indicators of pollution in the same way that coliform bacteria are.

II ALGAE AND ORGANIC POLLUTION

A Heavy pollution may tend to limit various kinds of algae to certain zones in the affected area.

B These zones are distinguished according to the degree of change which has occurred in the organic wastes. One set of names for these zones includes the Polysaprobic, alpha-mesosaprobic, beta-mesosaprobic and oligosaprobic.

C A few "pollution" algae are common in the first two zones. Many algae are common in and often limited to one or both of the last two zones.

D Some workers have listed separately those algae indicative of each of the four zones.

III REASONS FOR SELECTIVITY OF POLLUTANTS TO ALGAE

A Certain components of wastes are chemicals toxic to some algae but not to others.

B Wastes may have physical effects on certain algae. May cause plasmolysis, change in rate of absorption of nutrients, etc.

C Wastes may reduce available light, increase the water temperature, and cover up the areas for attachment to rocks.

D Wastes may prevent algal respiration at night by reducing the DO of water.

E Wastes may stimulate other organisms at the expense of certain algae.

F Products of waste decomposition may act as powerful growth stimulants for certain algae.

IV ALGAE AS INDICATORS OF POLLUTION

A Selection of list of "pollution" algae follows an evaluation of the kinds reported in published reports by numerous workers as relatively prominent in, or representative of, the polysaprobic and alpha-mesosaprobic zones in a stream polluted with sewage. It includes also other conditions or areas approximating these zones.

B A total list of more than 1000 kinds of algae has been compiled to date.

1 In order to tabulate the information, an arbitrary numerical value is allotted to each author's record of each pertinent alga.

2 The algae are then arranged in order of decreasing emphasis by the authors as a whole.

VI SOME GENERA AND SPECIES OF ALGAE HIGH ON THE LIST ARE AS FOLLOWS:

A Genera: Oscillatoria, Euglena, Navicula, Chlorella, Chlamydomonas, Nitzschia, Stigeoclonium, Phormidium, Scenedesmus, Ankistrodesmus, Phacus.

B Species: Euglena viridis, Nitzschia palea, Oscillatoria chlorina, Oscillatoria limosa, Oscillatoria tenuis Scenedesmus quadricauda, Stigeoclonium tenue, Synedra ulna and Pandorina morum.

VII SOME ALGAE REPRESENTATIVE OF CLEAN WATER ZONES IN STREAMS:

Chrysococcus rufescens, Cocconeis placentula, Entophysalis lemaniae, and Rhodomonas lacustris.

VIII RELIABILITY IN USE OF INDICATORS DEPENDS IN PART UPON ACCURATE IDENTIFICATION OF SPECIMENS

REPRESENTATIVE LITERATURE

1 Brinley, F.J. Biological Studies. Ohio River Pollution Survey. I. Biological Zones in a Polluted Stream. II. Plankton Algae as Indicators of the Sanitary Condition of a Stream. Sewage Works Journal, 14:147-159. 1942.

2 Butcher, R.W. Pollution and Repurification as Indicated by the Algae. Fourth International Congress for Microbiology (held) 1947. Report of Proceedings. 1949.

3 Fjerdingstad, E. The Microflora of the River Moelleaa with Special Reference to the Relation of the Benthal Algae to Pollution. Folia Limnological Scandinavia. No. 5. 1950.

4 Fjerdingstad, E. Taxonomy and Saprobic Valency of Benthic Phytomicro-Organisms. Intern. Rev. Ges. Hydrobiol. 50:475-604. 1965.

5 Hawkes, H.A. The Biological Assessment of Pollution in Birmingham Streams. The Institute of Sewage Purification, Journal and Proceedings. 177-186. 1956.

6 Kolkwitz, R. Oekologie der Saprobien. Schriftenreiche des Vereins für Wasser-, Boden-, und Lufthygiene Berlin-Dahlem. Piscator - Verlage, Stuttgart.

7 Lackey, J.B. The Significance of Plankton in Relation to the Sanitary Condition of Streams. Symposium on Hydrobiology. University of Wisconsin. 311-328. 1941.

8 Liebmann, H. Handbuch der Frischwasser - und Abwasserbiologie. R. Oldenbourg, Munchen.

9 Palmer, C.M. Algae as Biological Indicators of Pollution. In Biological Problems in Water Pollution. Trans. of 1956 Seminar. Robert A. Taft Sanitary Engineering Center. 1957.

10 Palmer, C.M. The Effect of Pollution on River Algae. Annal. N.Y. Acad. Sci. 108:389-395. 1963.

11 Palmer, C.M. A Composite Rating of Algae Tolerating Organic Pollution. Jour. Phycology 5 (1):78-82. 1969.

12 Palmer, C.M. Algae in Water Supplies of the United States. In: Algae and Man, Ch 12, Plenum Press. N.Y. pp. 239-261. 1964.

13 Patrick, R. Factors Effecting the Distribution of Diatoms. Botanical Review. 14: 473-524. 1948.

14 Whipple, G.C., Fair, G.M. and Whipple, M.C. The Microscopy of Drinking Water, 4th ed. J. Wiley and Sons. New York. 1948.

This outline was prepared by C.M. Palmer, Aquatic Biologist, Cincinnati Water Research Laboratory, FWPCA.

POLLUTED WATER ALGAE

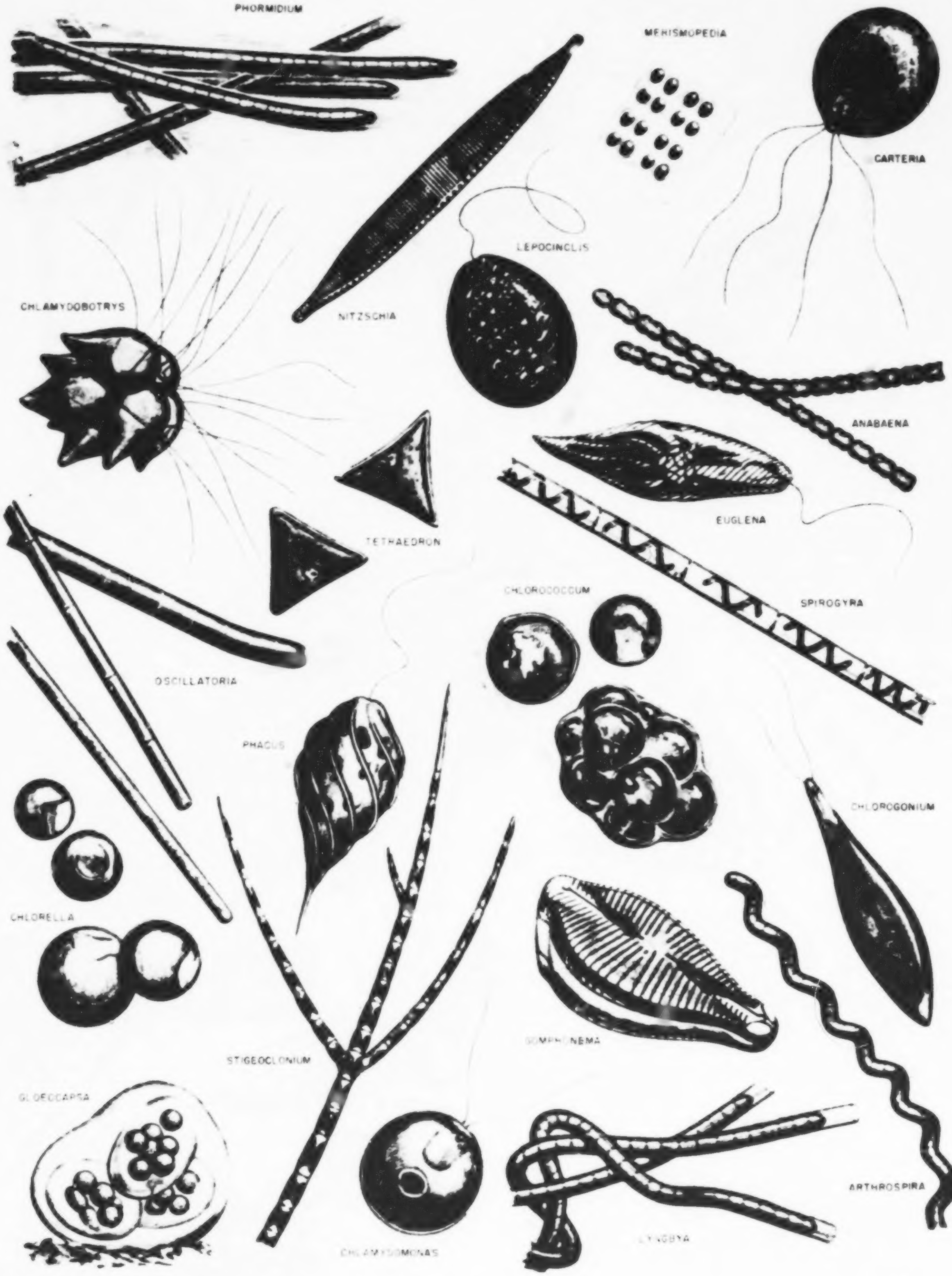
PHORMIDIUM
MERISMOPEDIA
CARTERIA
LEPOCINCLIS
CHLAMYDOBOTRYS
NITZSCHIA
ANABAENA
EUGLENA
TETRAEDRON
CHLOROCOCCUM
SPIROGYRA
OSCILLATORIA
PHACUS
CHLOROGONIUM
CHLORELLA
GOMPHONEMA
STIGEOCLONIUM
GLOEOCAPSA
ARTHROSPIRA
LYNGBYA
CHLAMYDOMONAS

PLATE 3

CLEAN WATER ALGAE

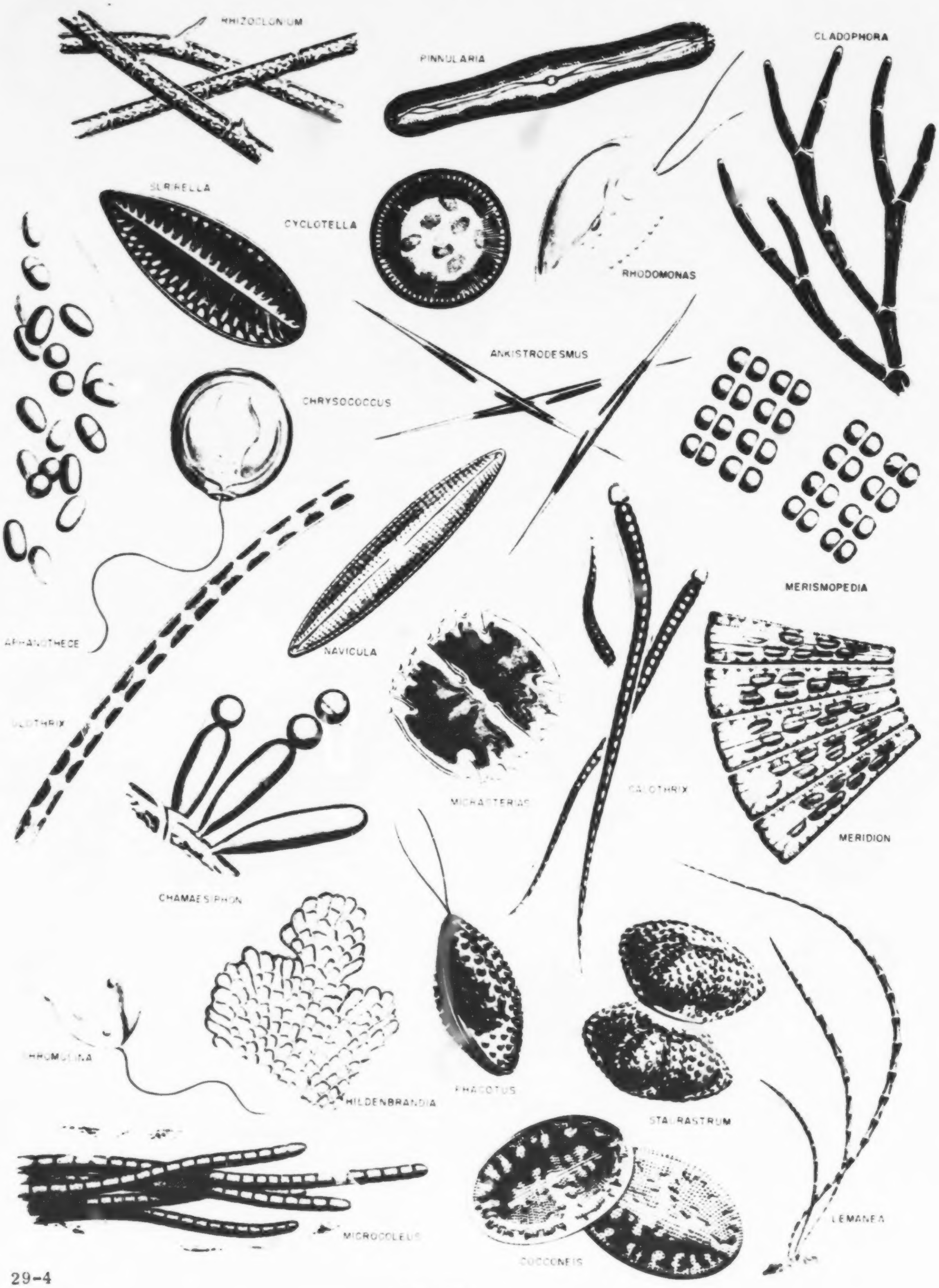
RHIZOCLONIUM
PINNULARIA
CLADOPHORA
SURIRELLA
CYCLOTELLA
RHODOMONAS
ANKISTRODESMUS
CHRYSOCOCCUS
MERISMOPEDIA
APHANOTHECE
NAVICULA
ULOTHRIX
MICRASTERIAS
CALOTHRIX
MERIDION
CHAMAESIPHON
CHROMULINA
HILDENBRANDIA
PHACOTUS
STAURASTRUM
LEMANEA
MICROCOLEUS
COCCONEIS

PLATE 4

ODOR PRODUCTION BY ALGAE AND OTHER ORGANISMS

I Most biological odors present in our water supplies are derived from algae, actinomycetes, and bacteria.

A The odor produced by algae and actinomycetes is generally the result of intracellular metabolic activity while the odor caused by bacteria usually results from extracellular enzymatic activity upon other organisms.

B The odors produced by actinomycetes are usually earthy while those produced by the algae are aromatic, grassy, and fishy.

II SOME SPECIES OF ALGAE CAUSING ODORS

A Diatoms

1 Asterionella (aromatic, fish)

2 Cyclotella (aromatic)

B Pigmented Flagellates

1 Synura (cucumber)

2 Dinobryon (fishy)

C Blue-green Algae

1 Anabaena (grassy, green corn, nasturtium)

2 Aphanizomenon (grassy, nasturtium)

D Green Algae

1 Chlorococcum (grassy)

III RESEARCH ON ALGAE ODORS

A Growing Algae for Odor Research

1 Obtaining unialgal bacteria-free cultures

a Plating out on semi-solid medium

b Single cell isolation

c Use of antibiotics

d Exposure to ultra-violet light

2 Determining nutritional requirements

a Inorganic salts

b Organic growth factors

B Methods of extracting odoriferous material from algal cultures

1 Distillation - steam and vacuum

2 Solvent extraction

3 Use of ion exchange resins

4 Freeze out methods

C Some Results of Research

1 Effect of culture age upon odor production

2 Effect of pH on odor intensity

3 Comparison of odor intensity in intact and broken cells

4 Groups of chemicals which may be responsible for causing algal odors

IV RESEARCH ON ACTINOMYCETE ODORS

A A number of actinomycetes were isolated from water and muds of rivers and lakes.

1 Large numbers were found to be present in muds, while there were relatively few in the water.

2 Most species belonged to the Streptomyces and a few to the Micromonospora.

B Extraction of Odoriferous Material

1 Streptomyces griseoluteus was used in this work.

a Cultured in a defined medium

(1) Cultures have threshhold odor of 20,000 to 50,000

2 Primary extraction was by distilling the culture at 100°C at atmospheric pressure.

a Distillation of 10% of the culture volume resulted in 90% odor removal.

3 Odor was further concentrated by two methods

a Ether extraction of the distilling off of the ether in vacuo.

(1) Resulted in yellowish brown concentrate having a threshold odor of approximately 6 billion.

b Absorption on activated carbon followed by elution of material with chloroform

C Effect of Activated Carbon in Removing the Earthy Odor

1 The odor is practically eliminated by 10 ppm carbon.

D Effect of Chlorine on Odor

1 Chlorine does not eliminate the odor but does not intensify the odor.

E Soil perfusion Tests

1 Conducted to determine the extent to which actinomycetes impart odors to a water environment.

REFERENCES

1 Fogg, G.E., "The Metabolism of Algae", John Wiley and Sons, Inc., New York, N. Y., 1953.

2 Fox, Leo, "Microscopic Organisms in Drinking Water", Taste and Odor Journal, Vol. 19, No. 10, 1953.

3 Palmer, C. M., and Tarzwell, C. M., "Algae of Importance in Water Supplies", Public Works Magazine, 1955.

4 Whipple, G. C., Fair, G. M., and Whipple, M. C., "The Microscopy of Drinking Water", Fourth Edition, John Wiley and Sons, Inc., New York, N.Y., 1948.

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PLANKTON IN OLIGOTROPHIC LAKES

I INTRODUCTION

The term oligotrophic was taken from the Greek words oligos -- small and trophein -- to nourish, meaning poor in nutrients. Lakes with low nutrient levels have low standing crops of plankton. The term is now commonly applied to any water which has a low productivity, regardless of the reason.

II PHYSICAL AND CHEMICAL CHARACTERISTICS OF OLIGOTROPHIC LAKES*

A Very deep; high volume to surface ratio

B Thermal stratification common; volume of the hypolimnium large compared to the volume of the epilimnion

C Maximum surface temperature rarely greater than 15°C

D Low concentrations of dissolved minerals and organic matter.

- 1 Phosphorus, less than 1 microgram per liter
- 2 NO_3-Nitrogen, less than 200 micrograms per liter

E Dissolved oxygen near saturation from surface to bottom

F Water very transparent, Secchi disk readings of 20-40 meters are common

G Color dark blue, blue-green, or green

III PLANKTON

A Quantity

- 1 Standing crop very low
  - a Ash-free weight of plankton, less than 0.1 mg per liter (compared to 1 mg per liter or more in eutrophic lakes).
  - b Chlorophyll, 1 mg per M^3 or less
  - c Cells counts, less than 500 per ml
- 2 Zooplankton to phytoplankton volume ratio, 19:1.

B Quality

1 European biologists have found oligotrophic lakes to be dominated by Chlorophyta (usually desmids), chrysophyta (such as Dinobryon), and Diatomaceae (Cyclotella and Tabellaria). Eutrophic lakes are dominated by Synedea, Fragilaria, Asterionella, Melosira, blue-green algae, Ceratium, and Pediastrum. Nygaard devised several phytoplankton quotients based on these relationships

a Simple quotient

Number of species of

$$\frac{\text{Chlorococcales}}{\text{Desmidiaceae}} = \begin{array}{l}\text{if} < 1,\ \text{oligotrophic}\\ \text{if} > 1,\ \text{eutrophic}\end{array}$$

b Compound index

$$\frac{\text{Myxophyceae}+\text{Chlorococcales}+\text{Centrales}+\text{Eugleniaceae}}{\text{Desmidiaceae}} =$$

if <1, oligotrophic

if 1-2.5, mesotrophic

if > 2.5, eutrophic

c Diatom quotient

$$\frac{\text{Centrales}}{\text{Pennales}} = \begin{array}{l}\text{if } 0\text{-}0.2,\ \text{oligotrophic}\\ \text{if } 0.2\text{-}3.0,\ \text{eutrophic}\end{array}$$

2 Several lists of trophic indicators have been published:

Two are listed here

Teiling, Swedish Lakes

Oligotrophic	*Tabelleria flocculosa*
	Dactylococcopsis ellipsoideus
Mesotrophic	*Kirchneriella lunaris*
	Tetraeadon spp.
	Pediastrum spp.
	Fragilaria crotonensis
	Attheya zachariasii
	Melosira granulata
Eutrophic	*Aphanizomenon* spp.
	Anabaena flos-aquae
	Anabaena circinalis
Pronounced Eutrophy	*Microcystis aeruginosa*
	Microcystis viridis

Rawson, Canadian Lakes

Oligotrophic	*Asterionella formosa*
	*Melosira islandica**
	Tabellaria fenestrata
	Tabellaria flocculosa
	Dinobryon divergens
	Fragilaria capucina
	Stephanodiscus niagarae
	Staurastrum spp.
	Melosira granulata
Mesotrophic	*Fragilaria crotonensis*
	Ceratium hirundinella
	Pediastrum boryanum
	Pediastrum duplex
	Coelosphaerium naegelianum
	Anabaena spp.
	Aphanizomenon flos-aquae
	Microcystis aeruginosa
Eutrophic	*Microcystis flos-aquae*

Some discrepancies can be seen in the ranking of species in the lists. These may be the result of true differences in the composition of the plankton, or may be only apparent differences which resulted from different sampling methods. Many studies (e.g. those by Hilliard, Olive, and Rawson) have been based on netted samples, which may be highly biased because they contain little of the nannoplankton. Also, it is not uncommon to characterize populations on the basis of one or two samples collected during the summer months.

3 The dominant plankton in four oligotrophic North American lakes are listed below. The Great Slave Lake and Karluk Lake data are from netted samples taken during the summer, and monthly, respectively. The Lake Superior and Lake Tahoe data are from grab samples taken twice monthly, and quarterly, respectively.

The dominant diatoms are generally similar in the four lakes. Asterionella formosa and Fragilaria crotonensis are common to all. There are also some obvious differences. Melosira islandica, the dominant diatom in the Great Slave Lake and Lake Superior, is absent from Lake Tahoe and Karluk Lake. It was not found in Crater Lake by Sovereign (1958), in the Mountain lakes of Colorado by Olive (1955) or Brinley (1950), and does not occur in WPSS samples in streams west of the Great Lakes. Tabellaria is also absent from Lake Tahoe. It was reported in Colorado lakes by Olive, but was not abundant. Brinley makes no reference to it, and Sovereign indicated that it was rare in Crater Lake samples. It is apparent that the absence of these two diatoms from Lake Tahoe is not related to the lake.

Except for the absence of Keratella cochlearis from Lake Tahoe, the rotifer populations are very similar. Data on other segments of the zooplankton population are insufficient to permit comparison.

	Rawson, Great Slave Lake	USPHS, Lake Superior	Hilliard, Karluk Lake	WPSS, Lake Tahoe
Dominant Phytoplankton	Melosira islandica	Melosira islandica	Asterionella formosa	Fragilaria crotonensis
	Asterionella formosa	Tabellaria fenestrata	Tabellaria flocculosa	Synedra nana
	Dinobryon divergens	Cyclotella kutzingiana	Fragilaria crotonensis	Fragilaria construens
	Ceratium hirundinella	Melosira granulata	Cyclotella bodanica	Fragilaria pinnata
	Pediastrum boryanum	Melosira ambigua	Cymbella turgida	Nitzschia acicularis
	Tabellaria fenestrata	Asterionella formosa	Dictyosphaerium spp.	Asterionella formosa
	Cyclotella meneghiniana	Synedra nana	Sphaerocystis spp.	
	Fragilaria crotonensis	Scenedesmus spp.	Staurastrum spp.	
	Fragilaria capucina	Ankistrodesmus spp.		
	Synedra ulna	Dictyosphaerium spp.		
	Eunotia lunaris			
Dominant Zooplankton	Keratella cochlearis	Keratella cochlearis	Not reported	Kellicottia longispina
	Kellicottia longispina	Kellicottia longispina		Daphnia spp.
	Diaptomus tenuicaudatus			Diaptomus tyrelli
	Limnocalanus macrurus			Epischura nevadensis
	Senecella calanoides			
	Daphnia longispina			
	Bosmina obtusirostris			

REFERENCES

1 Brinley, F.J. 1950. Plankton population of certain lakes and streams in the Rocky Mountain National Park, Colorado. Ohio J. Sci. 50:243-250.

2 Hilliard, D.K., 1959. Notes on the phytoplankton of Karluk Lake, Kodiak Island, Alaska. Canadian Field-Naturalist 43:135-143.

3 Jarnefelt, H., 1952. Plankton als Indikator der Trophiegruppen der seen. Ann. Acad. Sci. Fennicae A.IV:1-29.

4 Knudson, B.M., 1955. The distribution of Tabellaria in the English Lake District. Proc. Int. Assoc. Limnol. 12:216-218.

5 Nygaard, G., 1949. Hydrobiological studies in some ponds and lakes II. The quotient hypothesis and some new or little known phytoplankton organisms. Klg. Danske Vidensk. Selsk. Biol. Skrifter 7:1-293.

6 Olive, J.R., 1955. Some aspects of plankton associations in the high mountains lakes of Colorado. Proc. Int. Assoc. Limnol. 12:425-435.

7 Rawson, D.S., 1953. The standing crop of net plankton in lakes. J. Fish. Res. Bd. Can. 10:224-237.

8 Rawson, D.S., 1956. The net plankton of Great Slave Lake. J. Fish. Res. Bd. Can. 13:53-127.

9 Rawson, D.S., 1956. Algal indicators of trophic lake types. Limnol. Oceanogr. 1:18-25.

10 Rodhe, W., 1948. Environmental requirements of fresh-water plankton algae. Symb. Bot. Upsal. 10:1-149.

11 Ruttner, F., 1953. Fundamentals of Limnology, 2nd ed., Univ. Toronto Press, Toronto.

12 Sovereign, H.E., 1958. The diatoms of Crater Lake, Oregon. Trans. Amer. Microsc. Soc. 77:96-134.

13 Teiling, E., 1955. Some mesotrophic phytoplankton indicators. Proc. Int. Assoc. Limnol. 12:212-215.

14 USPHS, 1962. National Water Quality Network, Annual Compilation of Data, PHS Publ. No. 663.

15 Welch, P.S., 1952. Limnology, 2nd ed., McGraw Hill Book Co., New York.

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THE EFFECTS OF POLLUTION ON LAKES

I INTRODUCTION

The pollution of lakes inevitably results in a number of undesirable changes in water quality which are directly or indirectly related to changes in the aquatic community.

A Industrial Wastes may contain the following:

1 Sewage

2 Dissolved organics--synthetics, food processing wastes, etc.

3 Dissolved minerals--salts, metals (toxic and nontoxic), pigments, acids, etc.

4 Suspended solids--fibers, minerals, degradable and non-degradable organics

5 Petroleum products--oils, greases

6 Waste heat

B The Materials in Domestic Wastes which affect Water Quality are:

1 Pathogenic fecal microorganisms

2 Dissolved nutrients: minerals, vitamins, and other dissolved organic substances

3 Suspended solids (sludge)--degradable and non-degradable organic materials

C Pollution and Eutrophication

The discharge of domestic wastes often renders the receiving water unsafe for contact water sports and water supplies. For example, some beaches on the eastern seaboard and in metropolitan regions of the Great Lakes are unfit for swimming because of high coliform counts. Other effects of domestic pollution include changes in the abundance and composition of populations of aquatic organisms.

1 As the nutrient level increases, so does the rate of primary production.

2 Shore-line algae and rooted aquatics become more abundant. For example, problems have been experienced with *Cladophora* and *Dichotomosiphon* along the shores of Lakes Ontario, Erie, and Michigan. These growths interfere with swimming, boating, and fishing, and cause odors when the organisms die and decay.

3 The standing crop of phytoplankton increases, resulting in higher counts and greater chlorophyll content. Increases in phytoplankton abundance may result in taste and odor problems in water supplies, filter clogging, high turbidity, changes in water color, and oxygen depletion in the hypolimnion.

4 Populations of fish and larger swimming invertebrates increase, based on the increase in basic food production.

5 Changes in dominant species

a Diatom communities give way to blue-greens. Toxic blue-greens may pose a problem.

b Zooplankton changes include replacement of *Bosmina coregoni* by *B. longirostris*.

c Trout and whitefish are replaced by perch, bass, and rough fish.

d Hypolimnion becomes anaerobic in summer; bottom sludge buildup results in loss of fish food organisms, accompanied by increase in density of sludgeworms (oligochaeta).

II HISTORICAL REVIEW

The cultural eutrophication of a number of lakes in Europe and America has been well documented.

A Zurichsee, Switzerland

1 1896 - sudden increase in Tabellaria fenestrata

2 1898 - sudden appearance of Oscillatoria rubescens which displaced Fragilaria capucina

3 1905 - Melosira islandica var. helvetica appeared

4 1907 - Stephanodiscus hantzschii appeared

5 1911 - Bosmina longirostris replaced B. coregoni

6 1920
1924 - O. rubescens occurred in great quantities

7 1920 - milky-water phenomenon; precipitation of $CaCO_3$ crystals (40μ) due to pH increase resulting from photosynthesis

8 Trout and whitefish replaced by perch, bass, and rough fish

B Hallwilersee, Switzerland

1 1897 - Oscillataria rubescens not observed up to this time

2 1898 - O. rubescens bloomed, decomposed, formed H_2S, killing off trout and whitefish

C Lake Windermere, England (core study)

1 Little change in diatoms from glacial period until recent times

2 Then Asterionella appeared, followed by Synedra

3 About 200 years ago, Asterionella again became abundant

4 Asterionella abundance ascribed to domestic wastes

D Finnish Lakes

Aphanizomenon, Coelosphaerium, Anabaena, Microcystis, are the most common indication of eutrophy.

TABLE 1 CHANGES IN PHYSIO-CHEMICAL PARAMETERS

Zurichsee, Switzerland

Parameter	Date	Value
Chlorides	1888	1.3 mg/l
	1916	4.9 mg/l
Dissolved organics	1888	9.0 mg/l
	1914	20.0 mg/l

		Max.	Min.
Secchi Disk	before 1910	16.8M	3.1M
	1905 - 1910	10.0M	2.1M
	1914 - 1928	10.0M	1.4M
Dissolved oxygen, at 100 M, mid-summer	1910 - 1930	Minimum	100% saturation
	1930 - 1942	"	9% saturation

E Linsley Pond, Connecticut

1 Species making modern appearance include *Asterionella formosa*, *Cyclotella glomerata*, *Melosira italica*, *Fragilaria crotonensis*, *Synedra ulna*

2 *Asterionella formosa* and *Melosira italica* were considered by Patrick to indicate high dissolved organics

3 *Bosmina coregoni* replaced by *B. longirostris*

F Lake Monona, Wisconsin

1 Began receiving treated sewage in 1920, developed blue-green algal blooms.

G Lake Washington, Washington

1 1940 - *Bosmina longirostris* appeared

2 1955 - *Oscillatoria rubescens* seen for the first time, and constituted 96% of phytoplankton, July 1

H Lake Erie

1 Phytoplankton counts at Cleveland have increased steadily from less than 500 cells/ml in the 1920's to over 1500 cells/ml in the 1960's

2 Abundance of burrowing mayflies (*Hexagenia* spp.) in Western Lake Erie decreased from $139/m^2$ in 1930, to less than $1/m^2$ in 1961.

I Lake Michigan

1 Milky water observed in south end, and in limnetic region in mid-1950's and again in 1967.

2 During the period 1965-1967 the Chicago water treatment plant has found it necessary to increase the carbon dosage from 23 lbs/mil gal to 43 lbs/mil gal, and the chlorine dosage from 20 lbs/mil gal to 25 lbs/mil gal.

3 Phytoplankton counts in the south end now exceed 10,000/ml during the spring bloom.

III FACTORS AFFECTING THE RESPONSE OF LAKES TO POLLUTION INCLUDE:

A Depth-surface area ratio: A large hypolimnion will act as a reservoir to keep nutrients from recirculating in the trophogenic zone during the summer stratification period. Rawson found an inverse relationship between the standing crop of plankton, benthos, and fish, and the mean depth.

B Climate: Low annual water temperatures may restrict the response of the phytoplankton to enrichment.

C Natural color or turbidity: Dystrophic (brown-water) lakes may not develop phytoplankton blooms because of the low transparency of the water.

IV TROPHIC LEVEL

Except in cases where massive algal blooms occur, the trophic status of lakes is often difficult to determine. Core studies are used to determine trends in diatom populations which might indicate changes in nutrient levels over an extended period of time.

V CONTROL OF POLLUTION

The success of efforts to arrest the eutrophication process, and where desirable, reduce the trophic level of a lake, will depend on a thorough knowledge of the nutrient budget.

A Significant quantities of nutrients may enter a lake from one or more of the following sources:

1 Rainfall

2 Ground water

TABLE 2 PARAMETERS COMMONLY USED TO DESCRIBE CONDITIONS

		Oligotrophic Condition
1	Transparency	$\geq$ 10 meters
2	Phosphorus	$\leq 1 \mu g/l$
3	NO_3 - Nitrogen	$\leq 200 \mu g/l$
4	Minimum annual hypolimnetic oxygen concentration	near 100% saturation
5	Chlorophyll	$\leq 1 mg/m^3$
6	Ash-free weight of seston	$\leq 0.1 mg/l$
7	Phytoplankton count	$\leq 500/ml$
8	Phytoplankton quotients	
	a $\frac{\text{number of species of Chlorococcales}}{\text{number of species of Desmids}}$	<1
	b $\frac{\text{Myxophycease+Chlorococcales+Centrales+Euglenaceae}}{\text{Desmidaceae}}$	<1
	c $\frac{\text{Centrales}}{\text{Pennales}}$	0 - 0.2
9	Phytoplankton species present (see outline on plankton in oligotrophic lakes).	

3 Watershed runoff

4 Shoreline domestic and industrial outfalls

5 Pleasure craft and commercial vessels

6 Waterfowl

7 Leaves, pollen, and other organic debris from riparian vegetation

B The supply of nutrients from "natural" sources in some cases may be greater than that from cultural sources, and be sufficient to independently cause a rapid rate of eutrophication regardless of the level of efficiency of treatment of domestic and industrial wastes.

C Many methods have been employed to treat the symptoms, reduce the eutrophication rate, or completely arrest and even reverse the eutrophication process.

1 Use of copper sulfate, sodium arsenite, and organic algicides: It is not economically feasible to use algicides in large lakes.

2 Addition of carbon black to reduce transparency. This is likewise frequently impractical.

3 Harvesting algae by foam fractionation or chemical precipitation.

4 Reducing nutrient supply by (a) removal of N and P from effluents, (b) diversion of effluents, and (c) dilution with nutrient-poor water.

D Examples of lakes where control has been attempted by reducing the nutrient supply, are:

1 Lake Washington, Seattle

The natural water supply for this lake is nutrient poor ($Ca = 8$ mg/l, $P \leq 5$ µg/l, $TDS = 76$ mg/l). Since the turnover time of the water in this lake is only three years, it was expected that diversion of sewage would result in a rapid improvement of water quality. Diversion began in 1963, and improvements were noticeable by 1965 - including an increase in transparency, and a reduction in seston, chlorophyll, and epilimnetic phosphorus.

TABLE 3

PHOSPHORUS REDUCTION IN LAKE WASHINGTON

Year	Maximum phosphorus in upper 10 meters (µg/l)
1963	70
1964	66
1965	63

2 Green Lake, Washington

The lake has a long history of heavy blooms of blue-green algae. Beginning in 1959, low-nutrient city water was added to the lake, reducing the concentration of phosphorus by 70% in the inflowing water. By 1966, the lake had been flushed three times. Evidence of improvement in water quality was noted in 1965, when Aphanizomenon was replaced by Gleotrichia.

3 Lake Tahoe

This lake is still decidedly oligotrophic. To maintain its high level of purity, tertiary treatment facilities were installed in the major sewage treatment plant, and construction is now underway to transport all domestic wastes out of the lake basin.

REFERENCES

1 Ayers, J.C. and Chandler, D.C., Eds. Studies on the environment and eutrophication of Lake Michigan. Special Report No. 30. Great Lakes Research Division, Institute of Science and Technology, University of Michigan, Ann Arbor. 1967.

2 Brezonik, P.L., Morgan, W.H., Shannon, E.E., and Putnam, H.D. Eutrophication factors in North Central Florida Lakes. University of Florida Water Res. Center. Pub. #5, 101 pp. 1969.

3 Carr, J.F., Hiltunen, J.K. Changes in the bottom fauna of Western Lake Erie from 1930 to 1961. Limnol. Oceanogr. 10(4):551-569. 1965.

4 Frey, David G. Remains of animals in Quaternary lake and bog sediments and their interpretation. Schweizerbartsche Verlagsbuchhandlung. Stuttgart. 1964.

5 Edmondson, W.T., and Anderson, G.C. Artificial eutrophication of Lake Washington. Limnol. Oceanogr. 1(1):47-53. 1956.

6 Fruh, E.G. The overall picture of eutrophication. Paper presented at the Texas Water and Sewage Works Association's Eutrophication Seminar, College Station, Texas. March 9, 1966.

7 Fruh, E.G., Stewart, K.M., Lee, G.F., and Rohlich, G.A. Measurements of eutrophication and trends. J.W.P.C.F. 38(8):1237-1258. 1966.

8 Hasler, A.D. Eutrophication of lakes by domestic drainage. Ecology 28(4):383-395. 1947.

9 Hasler, A.D. Cultural Eutrophication is Reversible. Bioscience 19(5): 425-443. 1969.

10 Herbst, Richard P. Ecological Factors and the Distribution of *Cladophora glomerata* in the Great Lakes. Amer. Midl. Nat. 82(1):90-98. 1969.

11 National Academy of Sciences. Eutrophication: Causes, Consequences, Correction. 661 pp. 1969. (Nat. Acad. Sci., 2101 Constitution Avenue, Washington, DC 20418, 13.50).

12 Neel, Joe Kendall. Reservoir Eutrophication and Dystrophication following Impoundment. Reservoir Fisheries Res. Symp. 322-332.

13 Oglesby, R.T. and Edmondson, W.T. Control of Eutrophication. J.W.P.C.F. 38(9):1452-1460. 1966.

14 Stewart, K.M. and Rohlich, G.A. Eutrophication - A Review. Publication No. 34, State Water Resources Control Board, The Resources Agency, State of California. 1967.

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APPLICATION OF BIOLOGICAL DATA

I ECOLOGICAL DATA HAS TRADITIONALLY BEEN DIVIDED INTO TWO GENERAL CLASSES:

A Qualitative - dealing with the taxonomic composition of communities

B Quantitative - dealing with the population density or rates of processes occurring in the communities

Each kind of data has been useful in its own way.

II QUALITATIVE DATA

A Certain species have been identified as:

1 Clean water (sensitive) or oligotrophic

2 Facultative, or tolerant

3 Preferring polluted regions (see: Fjerdinstad 1964, 1965; Gaufin & Tarzwell 1956; Palmer 1963, 1969; Rawson 1956; Teiling 1955)

B Using our knowledge about ecological requirements the biologist may compare the species present

1 At different stations in the same river (Gaufin 1958) or lake (Holland 1968)

2 In different rivers or lakes (Robertson and Powers 1967)

or changes in the species in a river or/lake over a period of several years. (Carr & Hiltunen 1965; Edmondson & Anderson 1956; Fruh, Stewart, Lee & Rohlich 1966; Hasler 1947).

C Until comparatively recent times taxonomic data were not subject to statistical treatment.

III QUANTITATIVE DATA: Typical Parameters of this type include:

A Counts - algae/ml; benthos/m^2; fish/net/day

B Volume - mm^3 algae/liter

C Weight - dry wgt; ash-free wgt.

D Chemical content - chlorophyll; carbohydrate; ATP; DNA; etc.

E Calories (or caloric equivalents)

F Processes - productivity; respiration

IV Historically, the chief use of statistics in treating biological data has been in the collection and analysis of samples for these parameters. Recently, many methods have been devised to convert taxonomic data into numerical form to permit:

A Better communication between the biologists and other scientific disciplines

B Statistical treatment of taxonomic data

C In the field of pollution biology these methods include:

1 Numerical ratings of organisms on the basis of their pollution tolerance

(saprobic valency: Zelinka & Sladecek 1964)

(pollution index: Palmer 1969)

2 Use of quotients or ratios of species in different taxonomic groups (Nygaard 1949)

3 Simple indices of community diversity:

a Organisms are placed in taxonomic groups which behave similarly under the same ecological conditions. The number of species in these groups found at "healthy" stations is compared to that found at "experimental" stations. (Patrick 1950)

b A truncated log normal curve is plotted on the basis of the number of individuals per diatom species. (Patrick, Hohn, & Wallace 1954)

c Sequential comparison index. (Cairns, Albough, Busey & Chanay 1968). In this technique, similar organisms encountered sequentially are grouped into "runs".

$$\text{SCI} = \frac{\text{runs}}{\text{total organisms examined}}$$

d Ratio of carotenoids to chlorophyll in phytoplankton populations:

OD_{430}/OD_{665} (Margalef 1968)

OD_{435}/OD_{670} (Tanaka, et al 1961)

e The number of diatom species present at a station is considered indicative of water quality or pollution level. (Williams 1964)

f $\dfrac{\text{number of species (S)}}{\text{number of individuals (N)}}$

g $\dfrac{\text{number of species (S)}}{\text{square root of number of individuals } (\sqrt{N})}$

h $\dfrac{S - 1}{\log_e N}$ (Menhinick 1964)

i $d = \dfrac{\Sigma n_i (n_i - 1)}{N (N - 1)}$ (Simpson 1949)

where n_i = number of individuals belonging to the i-th species, and

N = total number of individuals

j Information theory:

The basic equation used for information theory applications was developed by Margalef (1957).

$$I = \frac{1}{N} \log_2 \frac{N!}{N_a!\, N_b! \ldots N_s!}$$

where I - information/individual; $N_a, N_b \ldots N_s$ are the number of individuals in species a, b, ... s, and N is their sum.

This equation has also been used with:

1) The fatty acid content of algae (McIntire, Tinsley, and Lowry 1969)

2) Algal productivity (Dickman 1968)

3) Benthic biomass (Wilhm 1968)

REFERENCES

1 Cairns, J., Jr., Albough, D.W., Busey, F, and Chaney, M.D. The sequential comparison index - a simplified method for non-biologists to estimate relative differences in biological diversity in stream pollution studies. J. Water Poll. Contr. Fed. 40(9):1607-1613. 1968.

2 Carr, J.F. and Hiltunen, J.K. Changes in the bottom fauna of Western Lake Erie from 1930 to 1961. Limnol. Oceanogr. 10(4):551-569. 1965.

3 Dickman, M. Some indices of diversity. Ecology 49(6):1191-1193. 1968.

4 Edmondson, W.T. and Anderson, G.C. Artificial Eutrophication of Lake Washington. Limnol. Oceanogr. 1(1):47-53. 1956.

5 Fjerdingstad, E. Pollution of Streams estimated by benthal phytomicroorganisms. I. A saprobic system based on communities of organisms and ecological factors. Internat'l Rev. Ges. Hydrobiol. 49(1):63-131.1964.

6 Fjerdingstad, E. Taxonomy and saprobic valency of benthic phytomicroorganisms. Hydrobiol. 50 (4):475-604. 1965.

7 Fruh, E.G., Stewart, K.M., Lee, G.F. and Rohlich, G.A. Measurements of eutrophication and trends. J. Water Poll. Contr. Fed. 38(8):1237-1258. 1966.

8 Gaufin, A.R. Effects of Pollution on a midwestern stream. Ohio J. Sci. 58(4):197-208. 1958.

9 Gaufin, A.R. and Tarzwell, C.M. Aquatic macroinvertebrate communities as indicators of organic pollution in Lytle Creek. Sew. Ind. Wastes. 28(7):906-924. 1956.

10 Hasler, A.D. Eutrophication of lakes by domestic drainage. Ecology 28(4):383-395. 1947.

11 Holland, R.E. Correlation of Melosira species with trophic conditions in Lake Michigan. Limnol. Oceanogr. 13(3):555-557. 1968

12 Margalef, R. Information theory in ecology. Gen. Syst. 3:36-71. 1957.

13 Margalef, R. Perspectives in ecological theory. Univ. Chicago Press. 1968.

14 McIntire, C.D., Tinsley, I.J. and Lowry, R.R. Fatty acids in lotic periphyton: another measure of community structure. J. Phycol. 5:26-32. 1969.

15 Menhinick, E.F. A comparison of some species - individuals diversity indices applied to samples of field insects. Ecology 45:859. 1964.

16 Nygaard, G. Hydrobiological studies in some ponds and lakes. II. The quotient hypothesis and some new or little-known phytoplankton organisms. Klg. Danske Vidensk. Selsk. Biol. Skrifter 7:1-293. 1949.

17 Patten, B.C. Species diversity in net plankton of Raritan Bay. J. Mar. Res. 20:57-75. 1962.

18 Palmer, C.M. The effect of pollution on river algae. Ann. New York Acad. Sci. 108:389-395. 1963.

19 Palmer, C.M. A composite rating of algae tolerating organic pollution. J. Phycol. 5(1):78-82. 1969.

20 Patrick, R., Hohn, M.H. and Wallace, J.H. A new method for determining the pattern of the diatom flora. Not. Natl. Acad. Sci., No. 259. Philadelphia. 1954.

21 Rawson, D.S. Algal indicators of trophic lake types. Limnol. Oceanogr. 1:18-25. 1956.

22 Robertson, S. and Powers, C.F. Comparison of the distribution of organic matter in the five Great Lakes. in: J.C. Ayers and D.C. Chandler, eds. Studies on the environment and eutrophication of Lake Michigan. Spec. Rpt. No. 30, Great Lakes Res. Div., Inst. Sci. & Techn., Univ. Michigan, Ann Arbor. 1967.

23 Simpson, E.H. Measurement of diversity. Nature (London) 163:688. 1949.

24 Tanaka, O.H., Irie, S. Izuka, and Koga, F. The fundamental investigation on the biological productivity in the Northwest of Kyushu. I. The investigation of plankton. Rec. Oceanogr. W. Japan, Spec. Rpt. No. 5, 1-57. 1961.

25 Teiling, E. Some mesotrophic phytoplankton indicators. Proc. Intern. Assoc. Limnol. 12:212-215. 1955.

26 Wilhm, J. L. Comparison of some diversity indices applied to populations of benthic macroinvertebrates in a stream receiving organic wastes. J. Water Poll. Contr. Fed. 39(10):1673-1683. 1967.

27 Wilhm, J. L. Use of biomass units in Shannon's formula. Ecology 49:153-156. 1968.

28 Williams, L. G. Possible relationships between diatom numbers and water quality. Ecology 45(4):810-823. 1964.

29 Zelinka, M. and Sladecek, V. Hydrobiology for water management. State Publ. House for Technical Literature, Prague. 122 p. 1964.

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THE PROBLEM OF SYNTHETIC ORGANIC WASTES

I Sources of organic chemicals in water are varied and of differing complexity.(1)

A Natural pollutants, such as algae, actinomycetes, etc. contribute to organic pollution.

1 Tastes and odors associated with these materials are probably not merely a result of decomposition, but are closely associated with materials produced during the life cycle of the organisms and plants.

2 Discharge of nutrients in the form of phosphorus and nitrogen compounds from domestic or other wastes frequently stimulate the production of natural pollutants.

B Industrial wastes, due to the rate of population increase and industrial expansion, have made the problem of effective water treatment an acute one in many places.

1 The production of synthetic organic chemicals has risen steadily over the past years, representing many new and complex products - and of importance to us - new and complex wastes.

2 The ideal method of handling industrial waste is at its source.

a However, what is often considered good treatment, still results in materials present in sufficient quantities to affect the taste and odor of water.

b Many problems are caused by slug discharges, often accidental.

C Domestic wastes in various stages of treatment.

D Miscellaneous sources also contribute to the problem.

1 Wastes from private and commercial boats.

2 Chemicals applied to the land may be washed into streams.

3 Chemicals applied directly to water.

a Evaporation control

b Killing off rough fish

c Aquatic plant control

II Concentrations of organic chemicals in water, even in comparatively minor quantities may cause difficulties.

A Wastes may contain from a few mg/l to several hundred mg/l of organic contaminants.

B Surface waters may contain from a few μg/l of organics to several mg/l.

1 Some of the chemicals isolated from water, along with the concentrations which can be detected by odor, are:(2)

Substance	Concentration Detectable*, μg/l
Formaldehyde	50,000
Picolines	500 - 1,000
Phenolics	250 - 4,000
Xylenes	300 - 1,000
Refinery hydrocarbons	25 - 50
Petrochemical waste	15 - 100
Phenyl ether	13
Chlorinated phenolics	1 - 100

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*Concentrations were determined by taking the median of 4-12 observations.

III The damaging effects of organics in water are becoming more apparent.

A Taste and odor in water is usually the first noticed effect from organics. This is a serious public relations and economic problem; it also may be a health problem.

B Organic contaminants may interfere with coagulation, damage ion exchangers, and create chlorine and carbon demand.

C In the stream they may have adverse effects on aquatic forms that support higher aquatic life, cause off-flavors in fish flesh, or have direct toxic effects on fish.

IV The methods of study employed in the collection and identification of organic chemicals in water involve physical and chemical methods and instrumental analysis.

A The comparatively small amounts of organic materials may be concentrated by adsorption on activated carbon.

1 This carbon is then extracted with appropriate organic solvents, the solvent extract is taken to dryness, the weighed extract is subjected to solubility group separation, and these individual groups may then be analyzed by various methods.

2 Employing the above method on Ohio River water, the following results were obtained:(3)

Chemical Group	% of Total	TOC in μg/l	Relative Odor Contribution
Water solubles	20	860	23
Ether and water insolubles	22	110	200
Neutral	14	3	4,670
Amine	4	575	7
Weak acid	8	645	12
Strong acid	6	365	16
Amphoteric	10	$5,000^{+}$	--
Loss	16	--	--

B Chemical separation and analyses may be accomplished by means of column chromatography, formation by derivatives, gas chromatography, infrared and ultraviolet spectroscopy, x-ray diffraction, etc.

1 Specific organic chemicals recovered from river and drinking waters by these methods include: synthetic detergents (ABS), phenylether, phenol, DDT, aldrin, o-nitrochlorobenzene, α-conedendrin, and xylene.

V Some of the types of problems that may be attributed to organic wastes, and more specifically to problems of taste and odor, may be represented by the following examples:

A By applying the previously mentioned carbon adsorption method, the odor potential of organic pollutants and the dilution necessary to reduce this odor potential to a barely perceptible level has been determined:(4)

Industry Source	Conc. Required for Detectable Odor µg/l		Dilution Factor	
	$CHCl_3$ Sol. Org.	Total Org.	$CHCl_3$ Sol. Org.	Total Org.
Brewery	770	1,400	14	86
Chemical	28	32	11,000	14,000
Corn Refining	1,000	3,600	1.4	2.1
Meat Packing	1,200	3,600	92	140
Metal Fabrication	890	1,600	2.8	4.8
Paint	390	1,000	69	98
Pharmaceutical	290	340	10	32
Refinery	84	510	780	760
Soap	900	1,800	640	350

B Of all the organic pollutants that can affect the taste and odor of drinking water, phenol has been the most extensively studied.

1 The potential sources of this chemical, both natural and synthetic, have been discussed.(5)

2 The course of chlorination of phenol, a common method of treatment in the water plant, has been shown to proceed by a process which starts with the pure compound (in itself relatively tasteless) and proceeds through strong-tasting intermediates to tasteless end products.(6)

C The effects of petrochemical wastes(7) on water quality are becoming increasingly important; it has been predicted that by 1970 the petrochemical production on a tonnage basis may be equal to 41% of all chemicals.

1 The three principal groups of petrochemicals are the paraffins, the naphthenes, and the aromatics. From these, over 200 basic products are manufactured, having thousands of subordinate uses.

2 Correspondingly, more than 100 identifiable compounds have been found in waste streams from petrochemical processes.

BIBLIOGRAPHY

1 Middleton, F. M. Taste and Odor Sources and Methods of Measurement. Taste and Odor Control Journal. 26:1. 1960.

2 Middleton, F.M., Rosen, A.A., and Burttschell, R.H. Taste and Odor. Research Tools for Water Utilities. Jour. A.W.W.A. 50:21. 1958.

3 Anonymous. Objectionable Organic Contaminants in Water. San. Eng. Center Activ. Rep. No. 25, 1955.

4 Sproul, O.J., and Ryckman, D.W. The Significance of Trace Organics in Water Pollution. PCF 33:1188. 1961.

5 Hoak, R.D. The Causes of Tastes and Odors in Drinking Water. Water and Sewage Works. 104:243. 1957.

6 Burttschell, R.H., Rosen, A.A., Middleton, F.M., and Ettinger, M.B. Chlorine Derivatives of Phenol Causing Taste and Odor. Jour. A.W.W.A. 51:205. 1959.

7 Gloyna, E.F., and Malina, Jr., J.F. Petrochemical Wastes Effects on Water. Indus. Water & Wastes, 7: #5. 134. Sept.-Oct. 1962.

8 Baker, R.A. Problems of Tastes and Odors. WPCF. 33:1099. 1961.

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SIGNIFICANCE OF "LIMITING FACTORS" TO POPULATION VARIATION

I INTRODUCTION

A All aquatic organisms do not react uniformly to the various chemical, physical and biological features in their environment. Through normal evolutionary processes various organisms have become adapted to certain combinations of environmental conditions. The successful development and maintenance of a population or community depend upon harmonious ecological balance between environmental conditions and tolerance of the organisms to variations in one or more of these conditions.

B A factor whose presence or absence exerts some restraining influence upon a population through incompatibility with species requirements or tolerance is said to be a limiting factor. The principle of limiting factors is one of the major aspects of the environmental control of aquatic organisms (Figure 1).

II PRINCIPLE OF LIMITING FACTORS

This principle rests essentially upon two basic concepts. One of these relates organisms to the environmental supply of materials essential for their growth and development. The second pertains to the tolerance which organisms exhibit toward environmental conditions.

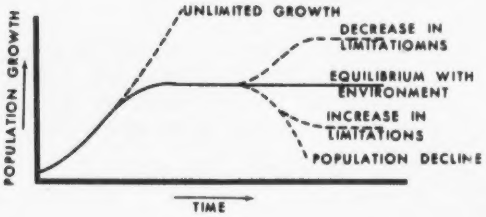

Figure 1. The relationships of limiting factors to population growth and development.

A Liebig's Law of the Minimum enunciates the first basic concept. In order for an organism to inhabit a particular environment, specified levels of the materials necessary for growth and development (nutrients, respiratory gases, etc.) must be present. If one of these materials is absent from the environment or present in minimal quantities, a given species will only survive in limited numbers, if at all (Figure 2).

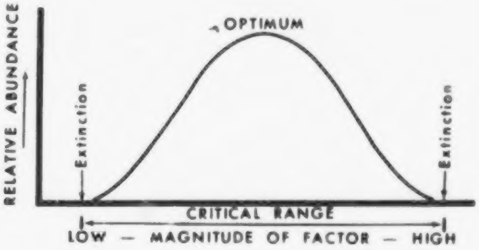

Figure 2. Relationships of environmental factors and the abundance of organisms.

1 The subsidiary principle of factor interaction states that high concentration or availability of some substance, or the action of some factor in the environment, may modify utilization of the minimum one. For example:

a The uptake of phosphorus by the algae Nitzchia closterium is influenced by the relative quantities of nitrate and phosphate in the environment; however, nitrate utilization appears to be unaffected by the phosphate (Reid, 1961).

b The assimilation of some algae is closely related to temperature.

c The rate of oxygen utilization by fish may be affected by many other substances or factors in the environment.

d Where strontium is abundant, mollusks are able to substitute it, to a partial extent, for calcium in their shells (Odum, 1959).

2 If a material is present in large amounts, but only a small amount is available for use by the organism, the amount available and not the total amount present determines whether or not the particular material is limiting (calcium in the form of $CaCO_3$).

B Shelford pointed out in his Law of Tolerance that there are maximum as well as minimum values of most environmental factors which can be tolerated. Absence or failure of an organism can be controlled by the deficiency or excess of any factor which may approach the limits of tolerance for that organism (Figure 3).

Minimum Limit of Toleration		Range of Optimum of Factors	Maximum Limit of Toleration	
Absent	Decreasing Abundance	Greatest Abundance	Decreasing Abundance	Absent

Figure 3. Shelford's Law of Tolerance.

1 Organisms have an ecological minimum and maximum for each environmental factor with a range in between called the critical range which represents the range of tolerance (Figure 2). The actual range thru which an organism can grow, develop and reproduce normally is usually much smaller than its total range of tolerance.

2 Purely deleterious factors (heavy metals, pesticides, etc.) have a maximum tolerable value, but no optimum (Figure 4).

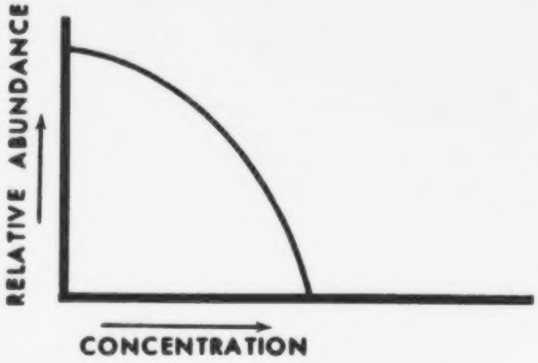

Figure 4. Relationship of purely harmful factors and the abundance of organisms.

3 Tolerance to environmental factors varies widely among aquatic organisms.

a A species may exhibit a wide range of tolerance toward one factor and a narrow range toward another. Trout, for instance, have a wide range of tolerance for salinity and a narrow range for temperature.

b All stages in the life history of an organism do not necessarily have the same ranges of tolerance. The period of reproduction is a critical time in the life cycle of most organisms.

c The range of tolerance toward one factor may be modified by another factor. The toxicity of most substances increases as the temperature increases.

d The range of tolerance toward a given factor may vary geographically within the same species. Organisms that adjust to local conditions are called ecotypes.

e The range of tolerance toward a given factor may vary seasonally. In general organisms tend to be more sensitive to environmental changes in summer than in other seasons. This is primarily due to the higher summer temperatures.

4 A wide range of distribution of a species is usually the result of a wide range of tolerances. Organisms with a wide range of tolerance for all factors are likely to be the most widely distributed, although their growth rate may vary greatly. A one-year old carp, for instance, may vary in size from less than an ounce to more than a pound depending on the habitat.

5 To express the relative degree of tolerance for a particular environmental factor the prefix eury (wide) or steno (narrow) is added to a term for that feature (Figure 5).

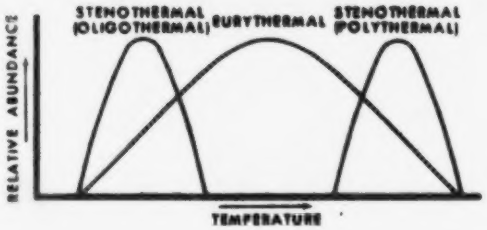

Figure 5. Comparison of relative limits of tolerance of stenothermal and eurythermal organisms.

C The law of the minimum as it pertains to factors affecting metabolism, and the law of tolerance as it relates to density and distribution, can be combined to form a broad principle of limiting factors.

1 The abundance, distribution, activity and growth of a population are determined by a combination of factors, any one of which may through scarcity or overabundance be limiting.

2 The artificial introduction of various substances into the environment tends to eliminate limiting minimums for some species and create intolerable maximums for others.

3 The biological productivity of any body of water is the end result of interaction of the organisms present with the surrounding environment.

III VALUE AND USE OF THE PRINCIPLE OF LIMITING FACTORS

A The organism-environment relationship is apt to be so complex that not all factors are of equal importance in a given situation; some links of the chain guiding the organism are weaker than others. Understanding the broad principle of limiting factors and the subsidiary principles involved make the task of ferreting out the weak link in a given situation much easier and possibly less time consuming and expensive.

1 If an organism has a wide range of tolerance for a factor which is relatively constant in the environment that factor is not likely to be limiting. The factor cannot be completely eliminated from consideration, however, because of factor interaction.

2 If an organism is known to have narrow limits of tolerance for a factor which is also variable in the environment, that factor merits careful study since it might be limiting.

B Because of the complexity of the aquatic environment, it is not always easy to isolate the factor in the environment that is limiting a particular population. Premature conclusions may result from limited observations of a particular situations. Many important factors may be overlooked unless a sufficiently long period of time is covered to permit the factors to fluctuate within their ranges of possible variation. Much time and money may be wasted on control measures without the real limiting factor ever being discovered or the situation being improved.

C Knowledge of the principle of limiting factors may be used to limit the number of parameters that need to be measured or observed for a particular study. Not all of the numerous physical, chemical and biological parameters need to be measured or observed for each study undertaken. The aims of a pollution survey are not to make and observe long lists of possible limiting factors but to discover which factors are significant, how they bring about their effects, the source or sources of the problem, and what control measures should be taken.

D Specific factors in the aquatic environment determine rather precisely what kinds of organisms will be present in a particular area. Therefore, organisms present or absent can be used to indicate environmental conditions. The diversity of organisms provides a better indication of environmental conditions than does any single species. Strong physio-chemical limiting factors tend to reduce the diversity within a community; more tolerant species are then able to undergo population growth.

REFERENCES

1 Odum, Eugene P. Fundamentals of Ecology, W. B. Saunders Company, Philadelphia. (1959)

2 Reid, George K. Ecology of Inland Waters and Estuaries. Reinhold Publishing Corporation, New York. (1961)

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NUTRIENTS: THE BASIS OF PRODUCTIVITY

I INTRODUCTION

A Nutrients of importance include macronutrients: those needed in large quantities, and micronutrients: those needed in small amounts.

B These nutrients are important because they promote biological responses which may interfere with some desired use of the water by man.

C Other factors (e.g. temperature, light) affect the use of these nutrients and should be considered in an evaluation of the effects of nutrients upon the ecosystem.

II Algae, bacteria, fungi and aquatic plants are the forms of life which nutrients affect most directly.

A Algae are of Several Types

1 Phytoplankton are small algae suspended in the water and form the basis of productivity in the aquatic environment.

2 Benthic algae are those forms anchored to substrates of rock and bottom materials.

3 Periphytic algal are those microscopic forms attached to submersed substrates.

B Aquatic plants are of several types. In general they may be referred to as rooted or floating forms.

C Heterotrophic bacteria are fungi which respond to organic nutrients introduced into water. Autotrophic bacteria may respond and grow due to inorganic nutrient sources.

III BIOLOGICAL LAWS

A Liebig's "law" of the minimum: the essential material available in amounts most closely approaching the critical minimum needed will tend to be the limiting factor.

B Shelford's "law" of tolerance: survival of an organism can be controlled by the quantitative or qualitative deficiency or excess with respect to any one of several factors which may approach the limits of tolerance for that organism.

C Q_{10} "law": with a temperature increase of 10 degrees centigrade metabolic processes (rates) are approximately doubled.

IV The process of photosynthesis is the fixation of the sun's energy with the production of organic matter by plants with chlorophyll.

A The general reaction is given below:

$$CO_2 + H_2O \xrightarrow{\lambda} CH_2O + O_2$$

B Chlorophyll contains basically C, O, H, N and Mg, and in general makes up about 5% of the dry weight of algal cells.

V MEASUREMENT OF PHOTOSYNTHESIS

A Oxygen production can be used as a measure of photosynthesis because for each mole of CO_2 reduced to organic carbon one mole of free oxygen is liberated.

1 The value of the molar O_2/CO_2 ratio has been found experimentally to vary within wide limits.

B CO_2 Assimilation

1 The CO_2 taken up by algae does not all originate from the dissolved gas. Some algae can use bicarbonate directly as a source of carbon.

2 Hence measurement of CO_2 uptake from water is a complicated problem which must consider pH, HCO_3^{-}, and $CO_3^{=}$ concentrations.

C Fixation of Carbon-14

1 The use of C^{14} as a tracer of C^{12} in plant metabolism and productivity estimation has been widely used since the early nineteen fifties.

2 In this method a known amount of C^{14} is added to the water and after a period of time the proportion of C^{14} in the plant cells to C^{14} added is found. The amount of carbon assimilated is then estimated from the following equation.

$$\frac{\text{activity of phytoplankton}}{\text{activity of } C^{14}O_3^{=} \text{ added}} (K) = \frac{\text{total carbon assimilated}}{\text{total carbon available}}$$

3 Where K is a constant relating to the slower uptake of C^{14}.

4 The total carbon available is determined chemically.

D Uptake of Mineral Nutrients

1 The measurement of depletion of nutrients in solution has been tried but found unreliable.

E Chlorophyll

1 The quality of chlorophyll present has been found to bear some relation to productivity but not a reliable one.

VI Nutrients of significance in the growth and production of algae and plants are discussed below.

A Carbon

1 Sources

a Gaseous CO_2

b HCO_3^{-}

c $CO_3^{=}$

d Other carbon compounds

2 Effects of the removal of carbon upon the water

a Lowered pH

b Deposition of $CaCO_3$

3 The quantity of carbon available is great and it usually is not a limiting factor.

B Nitrogen

1 Nitrogen can be taken up by most algae as either ammonium salts or as nitrates. Nitrites can also be used but a high concentration is usually inhibitory. Some blue green algae can fix atmospheric nitrogen. Certain algae varieties require supplementary amines, growth factors, etc.

2 The quantity of nitrogen in waters has definitely been shown to limit algal populations.

C Phosphorus

1 Phosphate seems to be the only inorganic source of this nutrient.

2 Limiting concentrations of P have been found to range from .01 ppm at a minimum and an inhibitory affect if P concentrations exceed 20 ppm.

3 Optimum concentrations have been found to range from .018 ppm to 15 ppm.

4 Storage of inorganic phosphate by algae has been demonstrated. The extent of this storage may reach 80% of the total phosphorus in algal cells.

D Silicon

1 Nutrient ratios in the algal cells of some areas have been found to be Si 23: N 16: P 1. It can be seen from this ratio that silicon is an important element in algal growth.

2 Silicon is especially important in the population growth of diatoms and may be the limiting growth factor in these populations.

E Inorganic micronutrients - Many elements are needed in very small quantities by algal cells. Some of these have a known function in algal metabolism; others do not.

1 Mg is a cation of major importance in the chlorophyll molecule.

2 Co is known to be necessary for vitamin B_{12}.

3 Mn is necessary for several enzyme systems.

4 Mo, V, Zn, and Cu are necessary but these functions are not as well known.

F Organic Micronutrients

1 Of 179 algal strains investigated about 40% required vitamin supplementation for optimum growth. Principal growth factors that were not synthesized in sufficient quantity are given as follows along with the percentage of the vitamin deficient strains showing marked productivity gain after supplementation:

a B_{12} addition increased growth on 80% of the strains.

b Thiamin addition increased growth on 53% of the strains.

c Biotin addition increased growth on 10% of the strains.

2 Algae can use and may require many organic compounds depending upon environmental conditions and the ability of the organism to synthesize required building blocks from mineral forms of C.N. & P. This is an area for continued investigation with many unappreciated or vaguely understood ecological factors.

VII PROBLEMS AND BENEFITS RESULTING FROM ALGAE PRODUCTION

A Problems to man may result when the total "primary production" by algae leads to an increase in the total organic content of the water that interferes with a desired use.

1 This may consist of a high algal population that produces a water with high turbidity, taste and odor, or other undesirable effect. High respiratory needs may lead to nocturnal oxygen deficit.

2 Certain algae may cause tastes and odors, clog filters; or otherwise interfere with potable water processing.

3 Death of large algal populations may lead to tastes and/or odors through bacterial decomposition. Oxygen deficits may result at any time of day in this process. Deposition of masses of organic sediment or sludge may be considerable.

4 Other problems might be cited.

B The primary production of algae can also serve as a supply of food to consumer organisms (animals), resulting in increased production at several (trophic) levels:

zoömicrobes, microinvertebrates, macroinvertebrates, fishes.

1 Earlier notation cited the release of oxygen during utilization of CO_2 during algal photosynthesis. This encourages fungal or bacterial breakdown of pollutants.

2 Photosynthesis occurs in the presence of adequate light and favorable conditions. In darkness, the cells continue to respire and may consume more oxygen than they produced because photosynthesis increases the organic load.

3 Photosynthesis tends to occur at the surface where light intensity is greatest. Poor vertical mixing would result in stratification of water supersaturated with oxygen over oxygen deficient water. Depending upon conditions, a significant fraction of the oxygen could be lost to the atmosphere.

4 Increased productivity may result in temporary reduction of the free dissolved nutrient level in the water but harvesting at some level is essential to prevent later recycle.

VIII CYCLE OF NUTRIENTS

A Once nutrients enter a body of water they are cycled through a food chain.

B Factors affecting this food chain (e.g. toxicity, removal) will affect the concentration and distribution of the nutrients.

ACKNOWLEDGEMENT:

This outline contains certain material submitted by F. J. Ludzack and H. W. Jackson.

REFERENCES

1 Golterman, H. L. and Clymo, R. S. Chemical Environment in the Aquatic Habitat (Proc. of an IBP - symposium, Amsterdam and Nieuwersluis Oct. 1966). 322 pp. (N. V. Noord-Hollandsche Uitgevers Maatschappij, Amsterdam. 8.95).

2 Lewin, Ralph A. Physiology and Biochemistry of Algae. Academic Press. 1962.

3 Odum, Eugene P. Fundamentals of Ecology. W. B. Saunders Co. 1959.

4 Odum, H. T. Primary Production in Flowing Waters. Limnology and Oceanography. 1(2):102-117. April 1956.

5 Ryther, John H. The Measurement of Primary Production. Limnology and Oceanography. 1(2):72-84. April 1956.

6 Verduin, Jacob. Primary Production in Lakes. Limnology and Oceanography. 1(2):85-91. April 1956.

7 Symposium: Factors That Regulate the Wax and Wane of Algal Populations. Inter. Assoc. of Theor. and Appl. Biol. Communications No. 19. 1971.

8 Likens, G. E. Nutrients and Eutrophication. The limiting-nutrient controversey. Am. Soc. Limnol. Ocean. Spec. Symp. Vol. 1. 1972.

9 Bartsch, A. F. Role of Phosphorus in Eutrophication. Ecol. Res. Ser. EPA - R3 - 72 - 001. 45 p. 1972.

10 Fitzgerald, G. P. Nutrient Sources For Algae and their Control. Water Pollution Cont. Res. Ser. 16010 EHR 08/71. EPA. 77 p. 1971.

This outline was prepared by Michael E. Bender, Biologist, Formerly with Training Activities, Ohio Basin Region, SEC.

ALGAE AND CULTURAL EUTROPHICATION

I INTRODUCTION

This topic covers a wide spectrum of items often depending upon the individual discussing the subject and the particular situation or objectives that he is trying to "prove". Since the writer is not a biologist, these viewpoints are "from the outside-looking in". Any impression of bias is intentional.

A Some Definitions are in Order to Clarify Terminology:

1 Eutrophication - a process or action of becoming eutrophic, an enrichment. To me, this is a dynamic progression characterized by nutrient enrichment. Like many definitions, this one is not precise; stages of eutrophication are classified as olig-, meso-, and eutrophic depending upon increasing degree. Just how a given body of water may be classified is open to question. It depends upon whether you look at quiet or turbulent water, top or bottom samples, season of the year, whether it is a first impression or seasoned judgement. It also depends upon the water use in which you are interested, such as for fishing or waste discharge. The transitional stages are the major problems - it is loud and clear to a trout fisherman encountering carp and scum.

2 Culture

Fostering of plant or animal growth; cultivation of living material and products of such cultivation, both fit. Some degree of control is implied but, the control may have limitations as well as advantages. Human cultural development has fostered human numbers successfully, but, has promoted rapid degradation of his natural environment.

3 Nutrients

A component or element essential to sustain life or living organisms. This includes many different materials, some in gross quantities - others in minor quantities. Deficiency of any one essential item make living impossible. Nutrients needed in large quantities include carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur and silica. N and P frequently are loosely considered as "the"nutrients because of certain solubility, conversion and "known" behavior characteristics.

4 Algae

A group of nonvascular plants, capable of growth on mineralized nutrients with the aid of chlorophyll and light energy - known as producer organisms, since the food chain is based directly or indirectly upon the organic material produced by algae.

B Now that we have "backed into" the title words via definitions, some of the ramifications of eutrophication, nutrient enrichment, and cultural behavior are possible.

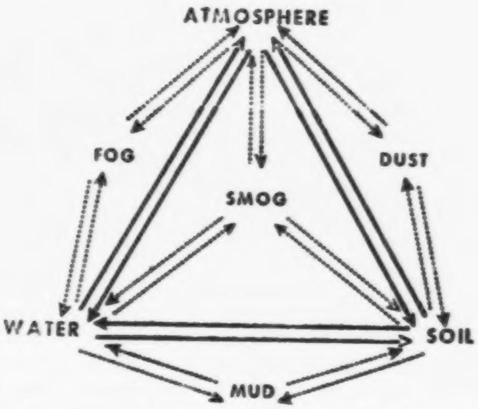
II NUTRIENTS INTERRELATIONSHIPS

A All nutrients are interchangeable in form, solubility, availability, etc. There are no "end" products. We can isolate, cover, convert to gas liquid or solid, oxidize, reduce, complex, dilute, etc. - some time, some place, that nutrient may recycle as part of cultural behavior.

1 Water contact is a major factor in recycle dynamics just as water represents two-thirds or more of cell

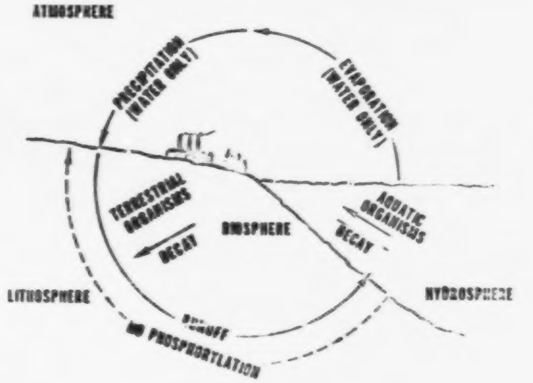
mass and appears to be the medium in which living forms started. Waste disposal interrelationships (Figure 1) suggests physical interrelationships of soil, air and water. The wet apex of this triangle is the basis for life. It's difficult to isolate water from the soil or atmosphere - water contact means solution of available nutrients.

WASTE DISPOSAL INTERRELATIONSHIPS


ATMOSPHERE
FOG
DUST
SMOG
WATER
SOIL
MUD

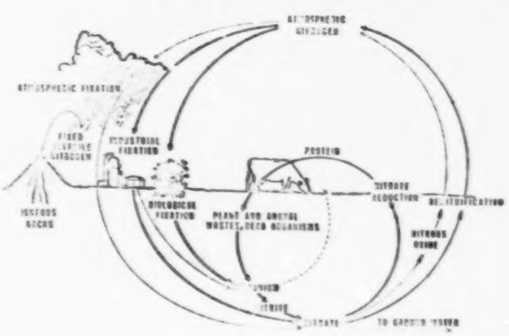
2 Figure 2 takes us into the biosphere (1) via the soluble element cycle. This refers mainly to phosphorus interchange. Phosphorus of geological origin may be solubilized in water, used by plants or animals and returned to water. Natural movement is toward the ocean. Less phosphorus returns by water transport. Phosphorus does not vaporize; hence, atmospheric transport occurs mainly as windblown dust. Man and geological upheaval, partially reverse the flow of phopshorus toward the ocean sink.

SOLUBLE ELEMENT CYCLE


ATMOSPHERE
PRECIPITATION (WATER ONLY)
EVAPORATION (WATER ONLY)
TERRESTRIAL ORGANISMS
DECAY
BIOSPHERE
AQUATIC ORGANISMS
DECAY
LITHOSPHERE
HYDROSPHERE
RUNOFF
AND PHOSPHORYLATION

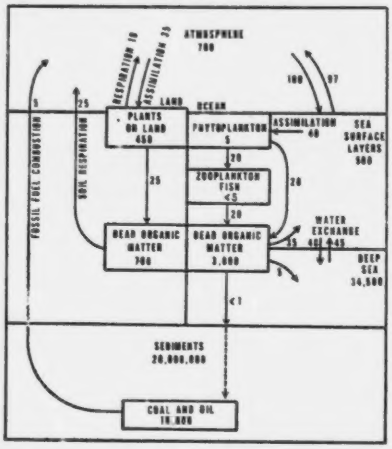
3 The nitrogen cycle starts with elemental nitrogen in the atmosphere. It can be converted to combined form by electrical discharge, certain bacteria and algae, some plants and by industrial fixation. Nitrogen gas thus may go directly into plant form or be fixed before entry. Denitrification occurs mainly via saprophytes. (Figure 3) Industrial fixation is a relatively new contribution to eutrophication.

NITROGEN CYCLE


ATMOSPHERIC NITROGEN
ATMOSPHERIC FIXATION
FIXED NITROGEN
IGNEOUS ROCKS
INDUSTRIAL FIXATION
PROTEIN
BIOLOGICAL FIXATION
PLANT AND ANIMAL WASTES, DEAD ORGANISMS
NITRATE REDUCTION
DENITRIFICATION
NITROUS OXIDE
AMMONIA
NITRITE
NITRATE
TO GROUND WATER

4 Carbon Conversions (Figure 4) show most of the carbon in the form of geological carbonate (1) but bicarbonate and CO_2 readily are converted to plant cell mass and into other life forms. Note the relatively small fraction of carbon in living mass.

CARBON CIRCULATION IN BIOSPHERE


ATMOSPHERE
700
RESPIRATION 10
ASSIMILATION 25
LAND
OCEAN
5
25
100
97
PLANTS ON LAND 450
PHYTOPLANKTON 5
ASSIMILATION 40
SEA SURFACE LAYERS 500
FOSSIL FUEL COMBUSTION
SOIL RESPIRATION
25
20
ZOOPLANKTON FISH <5
20
20
DEAD ORGANIC MATTER 700
DEAD ORGANIC MATTER 3,000
WATER EXCHANGE
35
40
45
5
DEEP SEA 34,500
<1
SEDIMENTS 20,000,000
COAL AND OIL 10,000

B Nutrient - Growth Relationships

Nutrient cycles could go on, but, life depends upon a mixture of essential nutrients under favorable conditions. Too much of any significant item in the wrong place may be considered as pollution. Since toxicity is related to chemical concentration, time of exposure and organism sensitivity, too much becomes toxic. If it happens to be too much growth, its a result of eutrophication. 'How much' is generally more important than the 'what'? Both natural and manmade processes lead to biological conversions, to pollution, to eutrophication and to toxicity. Man is the only animal that can concentrate, speed up, invent, or otherwise alter these conversions to make a collossal mess.

1 Life forms have been formulated in terms of elemental or nutrient components many times. The simplest is $C_5H_8O_2N$. A more complex formula is $C_{100}H_{76}O_{80}N_{20}Ca_6Cl_7P_2CuF_2SiMgMn_2K_2NaS_{21}Zn$. This includes 16 elements. More than 30 have been implicated as essential and they still would not "live", unless they were correctly assembled. As a nutrient Mnemonic H. COPKINS - - Mg(r)-CaFe-MoB does fairly well. It also indicates Iodine-I, Iron-Fe, Molybdenum-Mo, and Boron-B that were not included earlier.

2 The Law of Distribution states that "Any given habitat tends to favor all suitable species - any given species tends to be present in all suitable habitats." Selection tends to favor the most suitable species at a given place and time.

3 Liebigs Law of the Minimum, states that "The essential material available in amounts most closely approaching the critical minimum will tend to be the limiting growth factor "

4 Shelfords law recognizes that there will be some low concentration of any nutrient that will not support growth. Some higher concentration will stimulate growth. Each nutrient will have some still higher concentration that will be bacteriostatic or toxic. This has been discussed earlier but was considered in a different manner.

III BIOLOGICAL PROGRESSIONS

The biological "balance" appears to be a very transitory condition in cultural behavior. Man favors production. A steady state "balance" does not persist very long unless energy of the system is too low to permit significant growth. A progression of species where each predominent form thrives for a time, then is displaced by another temporarily favored group is usual. Yearly events in the lawn start with chickweed, then dandelion, plantain, crab grass, rag weed, etc., in successive predominence. Occasionally, more desirable grasses may appear on the lawn. Grass is a selected unstable "culture".

A Figure 3 shows a biological progression (2) following introduction of wastewater in an unnamed stream. Sewage or slime bacteria proliferate rapidly at first followed by ciliates, rotifers, etc.

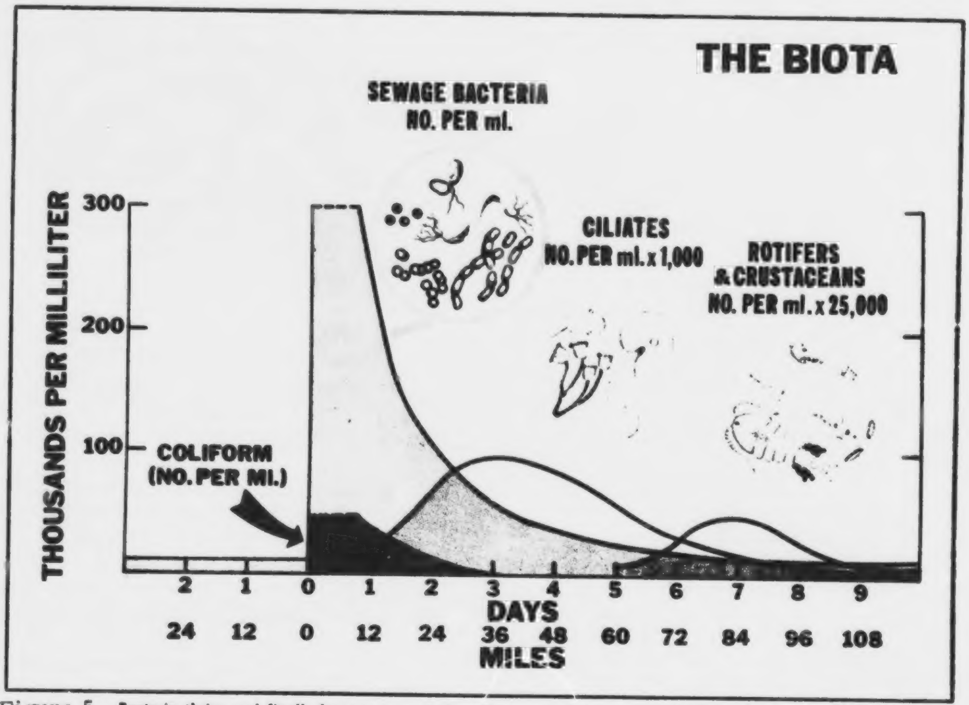


Figure 5. Bacteria thrive and finally become prey of the ciliates, which in turn are food for the rotifers and crustaceans.

B Figure 4 shows another progression of bottom dwelling larva. Here the sequence of organisms changes after wastewater introduction from aquatic insects to sludge worms, midges, sow bugs and then to re-establishment of insects.

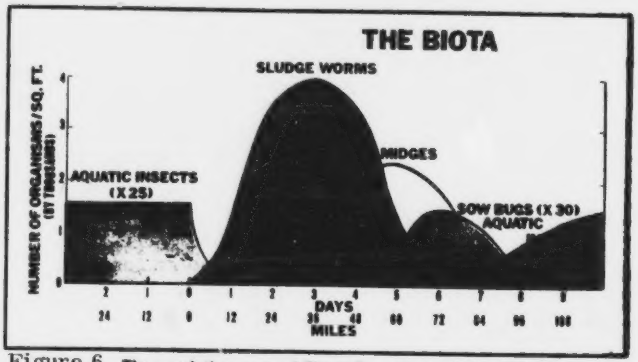
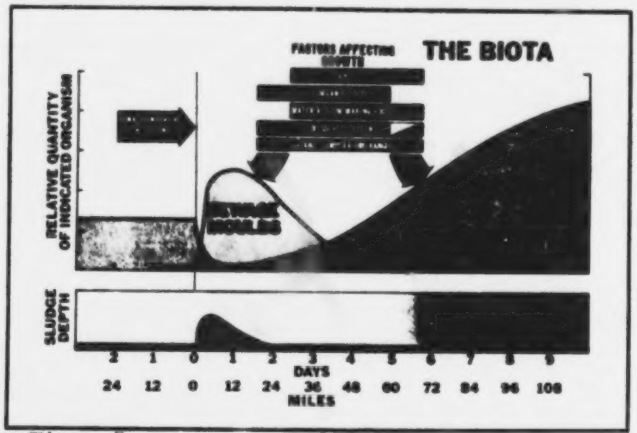


Figure 6. The population curve of Figure 7 is composed of a series of maxima for individual species, each multiplying and dying off as stream conditions vary.

C Another progression after waste introduction changes the biota from an algal culture to sewage moulds with later return to algal predominence.



● Figure 7 Shortly after sewage discharge, the moulds attain maximum growth. These are associated with sludge deposition shown in the lower curve. The sludge is decomposed gradually; as conditions clear up, algae gain a foothold and multiply.

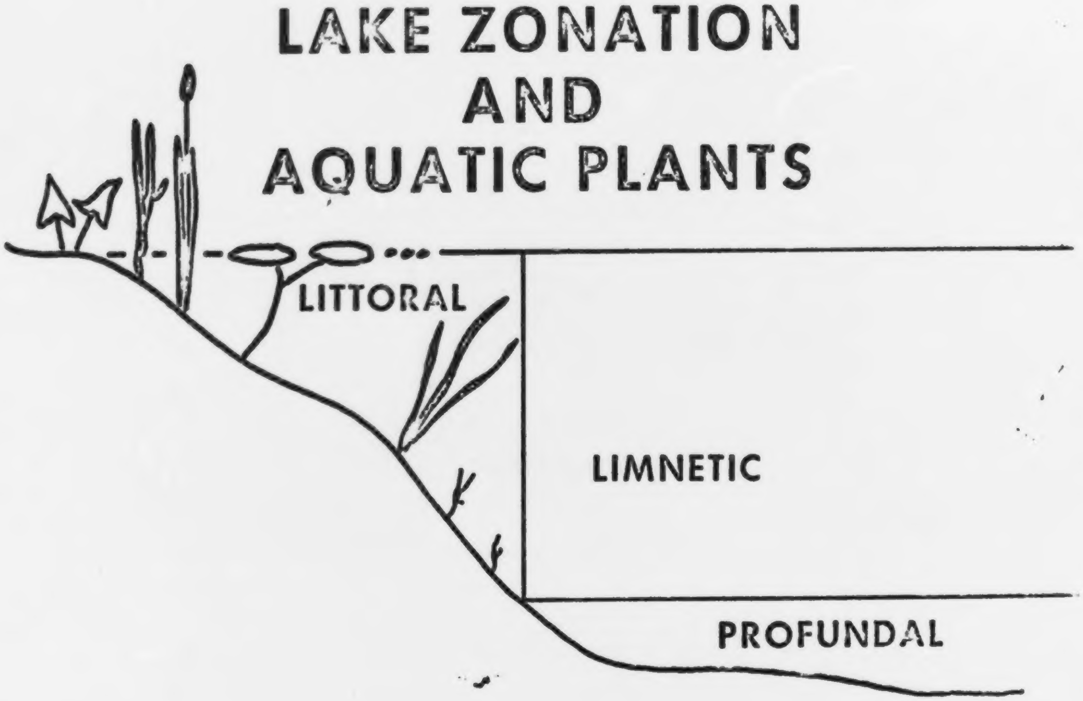
Figures 5, 6, and 7 are shown separately only because one visual would be unreadable with all possible progressions on it. There are progressions for fungi, protista, insect larvae, worms, fish, algae, etc. Each species will perform as it may perform. It it cannot compete successfully, it will be replaced by those that can compete under prevailing conditions at the time. Conditions shift rapidly with rapid growth.

IV The interactions of bacteria or fungi and algae (Figure 8) are particularly significant to eutrophication.

A The bacteria or the saprophytic group among them tend to work on preformed organic materials - pre-existing organics from dead or less favored organisms. Algal cells produce the organics from light energy chlorophyll and mineralized nutrients. This is a happy combination for both: The algae release the oxygen for use by the bacteria while the bacteria release the CO_2 needed by the algae.

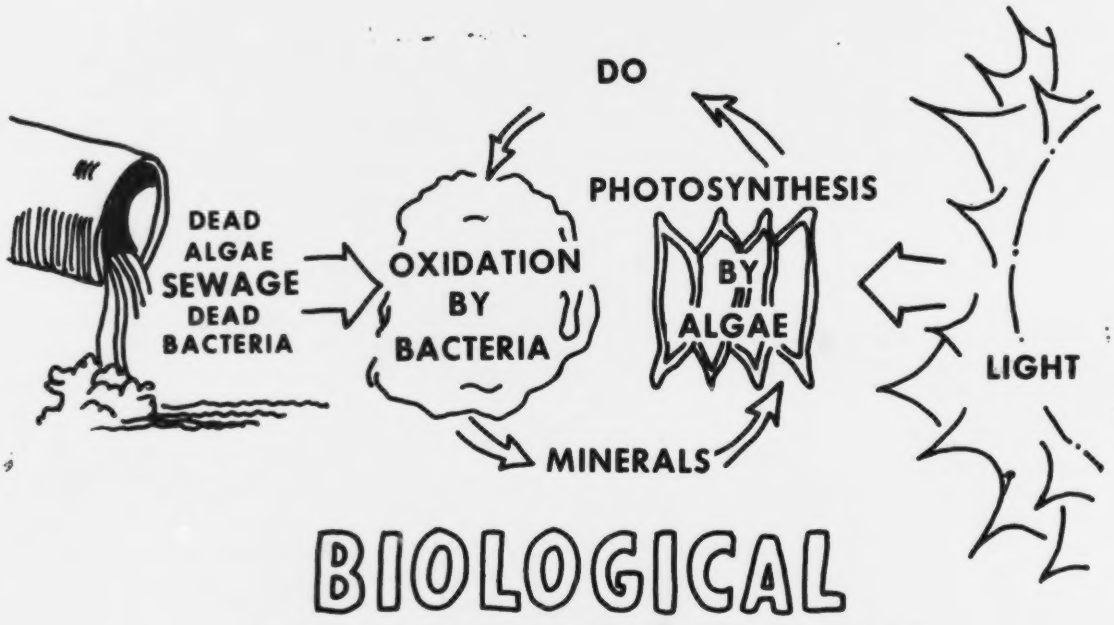
Since the algae also acquire CO_2 from the atmosphere, from wastewater and from geological sources, it always ends up with more enrichment of nutrients in the water - more enrichment means more growth and growing organisms eventually clump and deposit. The nature of growth shifts from free growth to rooted forms, starting in the shallows. Another progression occurs (Figures 9 and 10).

It is this relationship that favors profuse nuisance growth of algae below significant waste discharges. There is a tremendous pool of carbon dioxide available in geological formations and in the air. Transfer to the water is significant and encourages algal productivity and eventual eutrophication of any body of water, but, this does not occur as rapidly as when the water body is super saturated with CO_2 from bacterial decay of wastewater discharges or benthic deposits from them.



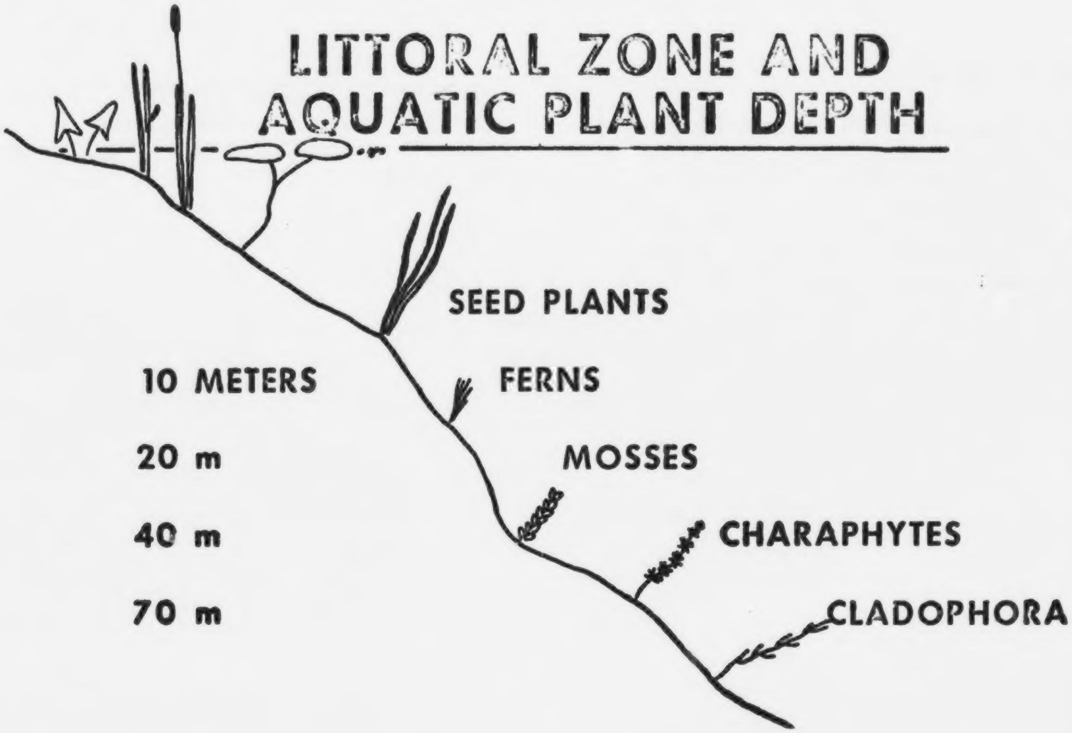
LAKE ZONATION AND AQUATIC PLANTS

LITTORAL
LIMNETIC
PROFUNDAL



BIOLOGICAL STABILIZATION

DO
PHOTOSYNTHESIS
DEAD ALGAE
SEWAGE
DEAD BACTERIA
OXIDATION BY BACTERIA
BY ALGAE
LIGHT
MINERALS



B Nitrogen and phosphorus are essential for growth. They also are prominently considered in eutrophication control. Algal cell mass is about 50% carbon, 15% nitrogen and approximately 1% phosphorus not considering luxury uptake in excess of immediate use. Phosphorus is considered as the most controllable limiting nutrient. It's control is complicated by the feedback of P from benthic sediments and surface wash. Phosphorus removal means solids removal. Good clarification is essential to obtain good removal of P. This also means improved removal of other nutrients- a major advantage of the P removal route. Both N & P are easily converted from one form to another; most forms are water soluble.

V SUMMARY

Control of eutrophication is not entirely possible. Lakes must eventually fill with benthic sediments, surface wash and vegetation. Natural processes eventually cause filling. Increased nutrient discharges from added activities grossly increase filling rate.

A We produce more nutrients per capita per day in the United States than in other nations and much more today than 100 years ago. More people in population centers accentuate the problem.

B Technology is available to remove most of the nutrients from the water carriage system.

1 This technology will not be used unless water is recognized to be in short supply.

2 It will not be used unless we place a realistic commodity value on the water and are willing to pay for cleanup for reuse purposes.

C Removal must be followed by isolation of acceptable gases to the atmosphere acceptable solids into the soil for reuse or storage. Water contact cannot be prevented, but it must be limited or the enrichment of the water body is hastened.

REFERENCES

1 A collection of articles on the Biosphere Sci. Am. 223:(No. 3). pp. 44-208. September 1970.

2 Bartch, A.E. and Ingram, W.M. Stream life and the Pollution Environment. Public Works 90:(No. 7) 104-110. July 1959.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, Water Programs Operations, EPA, Cincinnati, OH 45268.

CONTROL OF PLANKTON IN SURFACE WATERS

I PHILOSOPHICAL CONSIDERATIONS

A Plankton growths are as natural to aquatic areas as green plants are to land areas and respond to the same stimuli.

B Man is currently harnessing plankton forms to accomplish useful work.

1 For generation of oxygen

a Stabilization of waste waters in oxidation ponds

b Oxygen recovery from CO_2 in space travel

2 For augmentation of food supply

a Fish ponds

b Nitrogen fixation in rice growing

c Harvesting of algae for direct use as food

C A growing knowledge of the nutrient requirements of plankton organisms will lead to a more enlightened approach to ways and means of controlling their growth when desirable.

II CLASSICAL METHODS OF CONTROL

A Chemical

1 Inorganic

a Copper sulfate is used most extensively. It is most effective in preventive rather than curative treatment. It has long lasting effects in soft waters but is short-lived in hard waters due to precipitation of the Cu^{++} as a basic carbonate. The precipitated material accumulates in bottom muds and is toxic to certain benthal forms, some of which serve as important fish food.

Dosages are normally based on the alkalinity of the water. When alkalinity is < 40 mg/l, the recommended dosage is 0.3 mg/l of $CuSO_4 \cdot 5H_2O$ in total volume of water. When alkalinity is > 40 mg/l, recommended dosage is 2.0 mg/l in surface foot of water.

b Chlorine is preferable to copper sulfate in the control of certain forms of algae. However, it is difficult to apply in most instances and is very short-lived due to photo catalytic decomposition of $HClO \rightarrow HCl + O$.

2 Organic - Numerous organic compounds have been evaluated, especially in relation to control of blue-green algae. "Phygon", 2,3-dichloronaphthoquinone, has been field tested but is too specific in its action for general application.

III ECOLOGICAL CONTROL

A Theory - Ecological control is based upon the principle of preventing or restricting growth by limiting one or more of the essential requirements. This is an application of Liebig's Law of the Minimum. The logical avenues of control are as follows:

1 Elimination of light

2 Limiting nutrient materials

B Light - Many cities have solved the problem of plankton growths by the use of covered reservoirs, underground and elevated. Concurrently, they have solved contamination problems created by birds and atmospheric fallout. In open reservoirs, some success has been obtained by limiting light through the use of a film of activated carbon.

C Nutrients - Since phytoplankton (algae) serve as the base of the food chain, knowledge concerning their nutrient requirements is required for ecological control, when limitation of light is impractical. The nutrient requirements of phytoplankton are as follows:

1 Nature of - The major nutrients are:

- a Carbon dioxide
- b Nitrogen - ammonia and nitrates (also N_2)
- c Phosphorus - phosphates.

Minor nutrients are:

- d Sulfur - sulfates
- e Potassium
- f Trace inorganics - magnesium, iron, etc.
- g Trace organics-vitamins, amino acids

2 Sources of - See Fig. 1

- a Atmosphere
- b Groundwater - springs
- c Storm water or surface runoff
- d Waste waters - domestic sewage and industrial wastes.

3 Significance of each major nutrient

a Carbon dioxide - See Fig. 2
Usually present in great abundance. Rapidly replenished from atmosphere and bacterial decomposition of organic matter. No reasonable possibility of human control. Nature, however, does provide some control through elevated pH levels if carbon dioxide becomes depleted rapidly.

b Nitrogen - Like land plants, certain algal forms prefer nitrogen in the form of $NH_3(NH_4^+)$ and others prefer it in the form of NO_3^-. Both forms often become depleted during the growing season and reach maximum concentrations during the winter season. A level of 0.30 mg/l of inorganic nitrogen at the time of the spring turnover is considered to be the maximum permissible level.

All natural surface waters are saturated with nitrogen gas. This serves as a source of nitrogen for bacteria and algae capable of fixing it.

c Phosphorus - A key element in all plant and animal nutrition. The critical level is considered to be 0.01 mg/l at the time of the spring turnover. Phosphorus is needed to sustain nitrogen fixing forms.

D Practice Of

1 Exclusion of light - Practice well established in distribution system reservoirs but impractical on large storage reservoirs.

2 Nutrient limitation

a Control of surface run-off quality

1) Agricultural
2) Other

b Diversion of sewage plant effluents

1) Madison, Wisconsin
2) Detroit Lakes, Minnesota
3) Pending - State College, Pa.

c Tertiary treatment of sewage

1) Nitrogen removal - Because of the several forms is very difficult.

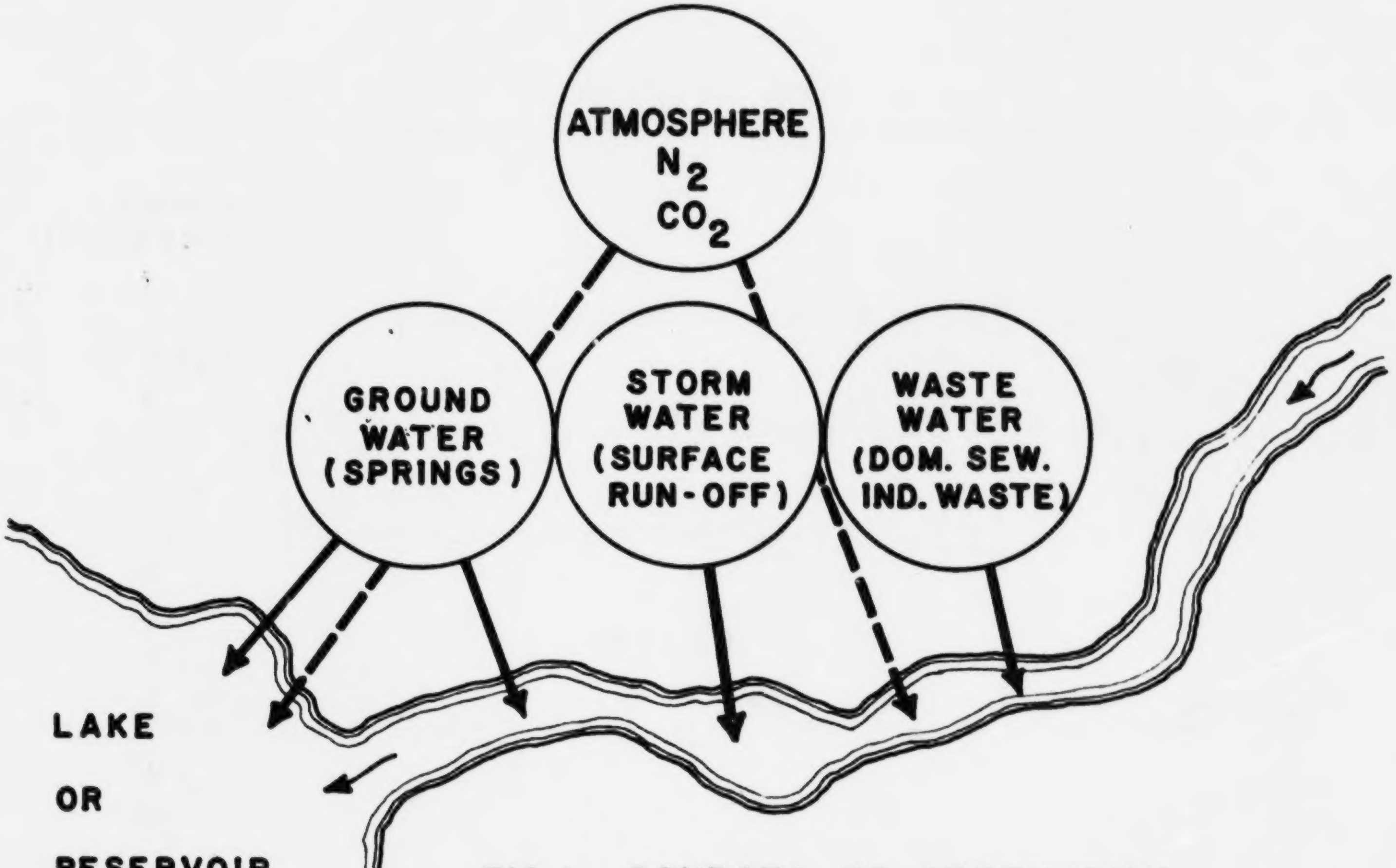

ATMOSPHERE
N_2
CO_2
GROUND WATER (SPRINGS)
STORM WATER (SURFACE RUN-OFF)
WASTE WATER (DOM. SEW. IND. WASTE)
LAKE
OR
RESERVOIR

FIG. 1 SOURCES OF FERTILIZING MATERIALS OF CONCERN IN SURFACE WATERS

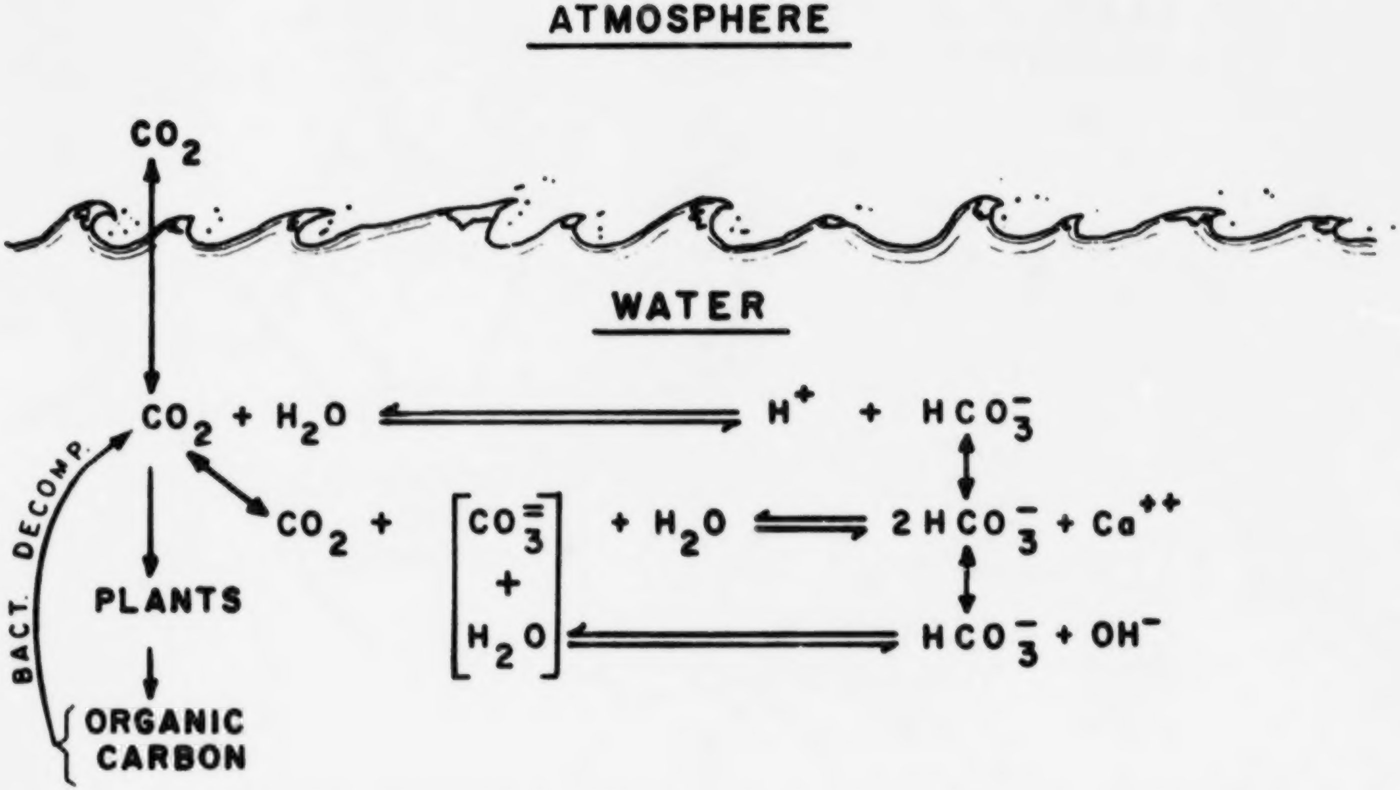

ATMOSPHERE
WATER
CO_2
$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$
$CO_2 + \begin{bmatrix} CO_3^= \\ + \\ H_2O \end{bmatrix} + H_2O \rightleftharpoons 2HCO_3^- + Ca^{++}$
$HCO_3^- + OH^-$
PLANTS
ORGANIC CARBON
BACT. DECOMP.

FIG. 2 CARBON DIOXIDE - BICARBONATE - CARBONATE - HYDROXIDE RELATIONSHIPS IN NATURAL WATERS

Also, may be unsuccessful in control unless phosphorus is controlled, too, because of nitrogen fixing forms.

2) Phosphorus removal - Phosphorus can be effectively removed by coagulation methods employing lime, alum or ferric salts. It is expensive and no one has proven its value beyond laboratory experiments.

d By Biological Engineering

Laboratory studies have shown that effluents essentially free of plant fertilizing elements can be produced by biological treatment of wastes with proper ratios of C to N and P.

3 Experiences

a Madison

b Detroit Lakes

c State College

d Lake Winnisquam, N.H.

E Practical Aspects

1 Diversion

2 Nutrient control

REFERENCE

Mullican, Hugh F. (Cornell Univ.) Management of Aquatic Vascular Plants and Algae. pp. 464-482. (in Eutrophication: Causes, Consequences, and Correction. Nat. Acad. Sci.) 1969.

This outline was prepared by C.N. Sawyer, Director of Research, Metcalf & Eddy Engineers, Boston, Massachusetts.

CONTROL OF INTERFERENCE ORGANISMS IN WATER SUPPLIES

I NECESSITY FOR DATA

A Information on the number, kinds, and effects of interference organisms in a particular water supply is essential for determining adequate control measures.

B Collection of the biological data should be on a regular routine basis.

C Interpretation of data requires information on relationship of number and kinds of organisms to the effects produced.

D It is generally more satisfactory to anticipate and prevent problems due to these organisms than it is to cope with them later.

II CONTROL IN RAW WATER SUPPLY

A Use of algicides

1 Application of an algicide is to prevent or destroy excessive growths of algae which occur as blooms, mats or a high concentration of plankton.

2 Algicide may be applied to control even low concentrations of certain algae such as Synura.

3 Copper sulfate is the only algicide in common use at present.

a Application may be by dusting, spraying or dissolving from a porous container over all or part of the water surface, or by continuous feeding of the algicide at the intake of the reservoir or pre-treatment basin.

b Effective dosage depends upon the Alkalinity and pH and temperature of the water and the amount and kinds of algae to be controlled. Bartch states that the following arbitrary dosages have been found to be generally effective and safe:

M.O. alkalinity > 50 p.p.m. = 2 p.p.m. in the surface foot of water only (5.4 pounds per acre).

M.O. alkalinity < 50 p.p.m. = 0.3 p.p.m. in total volume of water (0.9 pound per acre foot).

c Application of copper sulfate should be limited to the minimum effective dosage because of its corrosive properties, and its toxicity to fish and other aquatic animals.

4 Other algicides

a Promising types include inorganic salts, organic salts, rosin amines, antibiotics, quinones, substituted hydrocarbons, quaternary ammonium compounds, amide derivatives and phenols. Cuprichloramine which is a combination of copper, chlorine and ammonia, and also chlorine dioxide have shown promise as general algicides.

b For domestic water supplies they will have to be not only economically feasible but nontoxic to animal life and to green plants other than algae.

c Due to higher costs they will probably be used only when adequate plankton and algal records are kept, which would permit early localized treatment.

d Algicides selectively toxic to the particular algae of greatest significance would be useful.

5 Mechanical removal or spreading out to permit rapid drying may be the simplest way of handling massive growths which are detached and washed ashore.

6 Turbidity due to silt keeps down the plankton population. In shallow reservoirs, fish which stir up the bottom mud will aid in keeping turbidity due to silt high.

7 Provisions for keeping the amounts of nutrients to a minimum may be emphasized more in the future.

8 For new reservoirs, clearing the site

of vegetation and organic debris before filling will reduce the algal nutrients. Steep rather than gentle slopes will reduce the areas which allow marginal growths to occur.

III CONTROL IN TREATMENT PLANT

A Coagulation and sedimentation

1 When well regulated they often will remove 90 per cent or more of the plankton.

2 With low plankton counts, a coagulant aid may be required.

3 Frequent removal of sludge from the basins, especially during the warm seasons may help to reduce tastes and odors originating from decomposing organic sediment.

B Sand filtration

1 Both slow and rapid sand filters tend to reduce the plankton count of the effluent by 90 per cent or more, when well regulated.

2 For rapid filters, accumulated plankton can be removed or reduced by surface scraping and by back washing.

C Micro-straining

1 This involves the passing of the water through a finely woven fabric of stainless steel. All but the smaller plankton organisms tend to be removed from the water. It is being used in some treatment plants in England and elsewhere.

D Activated carbon

1 The slightly soluble, organic, taste and odor compounds tend to be readily adsorbed by the activated carbon. It is probably most often applied prior to coagulation, but may be used prior to filtration or in the raw water.

E Chlorination

1 Treatment with chlorine is practiced primarily to destroy pathogenic organisms. The dosages commonly used are toxic also to many algae and to some of the other groups of aquatic organisms. However, dead as well as living organisms are often capable of causing tastes and odors and of clogging filters.

F The depth and position of the intake for entrance of raw water into the treatment plant may determine the kinds and amount of plankton which will be drawn into the plant. Plankton algae generally are more concentrated near the surface of the water in lakes and reservoirs.

IV CONTROL IN DISTRIBUTION SYSTEM

A Maintenance of a chlorine residual controls the chlorine sensitive organisms.

B Other pesticides such as cuprichloramine have been used in attempts to control the resistant organisms such as worms, nematodes and copepod eggs.

C Flushing of infested portions of the system, especially dead ends may be practiced.

D Covering of treated water reservoirs to prevent the entrance of light will stop the growth of algae.

E Organisms associated with pipe corrosion are probably the most active when the water itself is corrosive.

F Mechanical cleaning of the distribution system may be an effective but expensive method of reducing infestations of attached organisms.

V SUMMARY

A Adequate control is dependent upon adequate procedures for detecting and recording of organisms.

B Control may involve the following:

1 Use of an algicide or pesticide.

2 Mechanical cleaning of distribution lines, settling basins, and filters, screens, intake channels and reservoir margins.

3 Modification of coagulation, filtration, chemical treatment or location of raw water intake.

4 Use of adsorbent, such as activated carbon, for taste and odor substances.

5 Modification of reservoir to reduce the opportunities for massive growths.

  a By covering treated water reservoirs

  b By increasing the depth of the water

  c By eliminating shallow marginal areas

  d By reducing the amount of fertilizing nutrients entering the reservoir

  e By encouraging a balanced development of the aquatic organisms

REFERENCE

Mackenthun, Kenneth M. The Practice of Water Pollution Biology. FWPCA. U.S. Dept. of Interior, Washington, DC. 1969.

This outline was prepared by C.M. Palmer, Former Aquatic Biologist, Biological Treatment Research Activities, Cincinnati Water Research Laboratory, FWPCA, SEC.

CASE PREPARATION AND COURTROOM PROCEDURE

I TYPES OF PROCEEDINGS IN WHICH WATER QUALITY EVIDENCE MAY BE USED

A Administrative Proceedings

1 Rule making

a Setting up of regulations having general application, e.g., stream classifications and implementation plan target dates

b Factors of safety and absolute prohibitions may be appropriate

2 Adjudications

a Determinations by agency having expertise with respect to particular discharge or discharger, e.g., approval of plans and specs and time schedule of a particular discharger

B Court Actions

1 Civil in behalf of state or federal government

a Actions to compel action or suspension of action - nuisance, health hazard, etc.,--including court action following federal conference --hearing procedure

b Violations of Water Quality Standards

c Violations of Effluent Standards or discharge permits

d Tort or contract actions relating to design and/or operation of treatment facilities

2 Criminal (dependent on content of applicable statutes).

a Discharge of specific materials

b Discharges from specific industries

c Littering

d Discharges harmful to fish and/or crustaceans

e Discharges harmful to specific types of receiving waters

f Discharges of poisons

NOTE--In some of these situations doing the act may constitute the violation; in others proof of intent or knowledge of effects may also have to be proved.

3 Private actions for damages or to compel action

a Alleged harm to plaintiff, e.g., pollution of stream killing animals

C Procedural Matters

1 See Attached sheet "Administrative and Court Proceedings" on Burden of proof, fact finding, and methods of presentation of evidence.

D Classes of Evidence - General Rules

1 Facts - direct

a The material was floating from the outfall.

2 Derived values - expert testimony - test results and/or opinion as to effects

a The D.O. was zero; the waterway was polluted; the plant can be built in 6 months.

3 Hearsay

a Joe told me

4 Relevancy

5 Admissibility vs. weight

a Even if admissible, the weight to be given is up to fact finder--credibility.

E Admissibility of Results of Sampling and Testing (Numbers)

1 Sampling

a Chain of custody

b Tags, etc.

c Containers

d Place and time

e Retention of samples (Proving that the sample represents what is at issue in the action (relevancy), that there has been no opportunity for tampering; and availability of portions for analysis by other side (non-transitory criteria)).

2 Analysis

a Who performed (Can identity of each participant be shown?)

b Admission through supervisor - custodian

c Scientific acceptance of method. Is there a particular method required to be used by the agency?

d Propriety of conduct

e Retention of bench cards and other indicia of results. (Your attorney can make arrangements to substitute copies for originals).

3 Tests

a Comparison with actual conditions

b Mathematical models - how can a computer be cross-examined?

F Admissibility of Expert Opinion on Causes and Effects

1 Who has special knowledge - and of what particular areas?

2 Indicators

3 Significance of numerical determinations or observations

4 Consistency with own prior publications and testimony

5 Have underlying facts been or need to be proved--first hand information of this and/or comparable situations.

6 Use of treatises

G Conduct on the Witness Stand

1 General

a On direct - know what counsel will ask and let him know generally what you will answer, but don't make it sound rehearsed.

b Use layman's language to extent possible.

c Listen to question and answer it to best of your ability.

d Speak so that court reporter, judge, jury, and counsel can hear you.

e Speak in language that will be understood; don't talk down.

f Answer only what you are asked --don't volunteer; however, answer with precision.

g There is nothing wrong with asking to have a question repeated or rephrased.

h There is nothing wrong with saying that you consulted with your attorney before you testified, but beware of the question "Did Mr. X tell you what to say?"

- i There is nothing wrong with thinking out your answer before responding.
- j You are not expected to know all the answers--if you do not know, admit it.
- k Don't attempt to answer questions outside your area of personal knowledge (hearsay) or beyond your expertise. (Your may be an expert on conducting laboratory tests, but not on epidimeological inferences from results).
- l Don't try to answer before the judge rules on objection.
- m Show that you are an impartial dispenser of information and/or opinion, not a protagonist.
- n Don't be afraid to admit what may appear to be damaging.

2 If you are testifying as an expert:

- a Establish qualifications -- give information relevant to your area of expertise -- educational (including this course?), work, publications, number of times you have testified previously.
- b Differentiate between physical facts (measurements and observations) and opinion (derived values).
- c Be prepared to discuss theory (including assumptions) instruments used, techniques(including choice of a particular technique), physical limitations and errors, interferences.
- d If experiments were conducted, be able to justify both as to theory and relevancy to this litigation.
- e If you're being paid to testify, admit it.

3 Scientific personnel as advisers to counsel:

- a Review and refamiliarize self with materials before you discuss with your attorney.
- b Be in a position to present all facts known to you simply and concisely: Who, What, When, Where, and Why, How.
- c Don't overlook facts and/or test results because you don't think they're important. Let attorney decide what he needs.
- d Use of standard report forms
- e Ability to recommend additional witnesses with needed specialized knowledge
- f Ability to aid in cross-examination of other side's experts and reconcile opinions and/or results
- g Be candid - sometimes better not to start a lawsuit or accept a settlement than lose in the end.

H Non-Verbal Presentation of Evidence

1 Exhibits - including photographs

2 Summaries

3 Business and/or government records

- a Prepared contemporaneously and in usual course of activities

4 Pre-prepared direct examination

- a Usually limited to actions before ICC, FPC, and other federal agencies.

I Criminal Procedure

1 Privilege Against Self Incrimination (available only to persons)

- a Warning and suspects
- b Effect of duty to report spills

  - c Effect of duty to obtain license or permit and/or furnish operating reports
  - d Immunity from prosecution

- 2 Double Jeopardy
- 3 Unreasonable search and seizure
  - a Available to persons and corporations
- 4 Procedures and need for arrest and search warrants --possible cause

This outline was prepared by David I. Shedroff, Enforcement Analyst, Office of Enforcement and General Counsel, Cincinnati Field Investigations Center, 5555 Ridge Avenue, Cincinnati, OH 45268.

Descriptors: Courtroom Procedure, Law Enforcement, Legal Aspects, Sampling, Water Analysis, Water Pollution Control, Water Quality Standards

Administrative & Court Proceedings, and Excerpts from Revised Draft of Proposed Rules of Evidence for the United States Courts can be found on the following pages.

ADMINISTRATIVE & COURT PROCEEDINGS

Court or Agency	Fact Finder	Burden of Proof	Comments
State Pollution Control Agency	Agency	As per statute - usually weight of evidence.	Hearing may be conducted by hearing examiner, agency member, or full agency. Appeal may be on facts and law or law alone, depending on statute.
Rule making-adjudication			
Federal Water Pollution Control Act			
Conference	Head of agency		Reports acceptable.
Hearing	Hearing Board		Specific testimony.
Court	Judge		Uses prior material, and may take additional testimony.
Court			
Civil Case -- - for money only	Judge or jury	Weight of evidence	
- injunction			
preliminary or temporary	Judge	Must show immediate harm or danger.	Must also show likelihood of success at final hearing - bond required for non-government plaintiff.
permanent	Judge	Usually clear and convincing.	"Balance Equities"
- administrative appeal	Judge - whether "arbitrary and capricious" or substantial evidence.		Sometimes have complete new trial.
Criminal case includes penalties	Jury unless waived.	Beyond reasonable doubt.	Proof of intent may be required.

Excerpts from Revised Draft of Proposed
RULES OF EVIDENCE FOR THE UNITED STATES COURTS

GENERAL PROCEDURES

Rule 102.
PURPOSE AND CONSTRUCTION

These rules shall be construed to secure fairness in administration, elimination of unjustifiable expense and delay, and promotion of growth and development of the law of evidence to the end that the truth may be ascertained and proceedings justly determined.

Rule 101.
PRELIMINARY QUESTIONS

(a) Questions of Admissibility Generally. Preliminary questions concerning the qualification of a person to be a witness, the existence of a privilege, or the admissibility of evidence shall be determined by the judge, subject to the provisions of subdivision (b). In making his determination he is not bound by the rules of evidence except those with respect to privileges.

(b) Relevancy Conditioned on Fact. When the relevancy of evidence depends upon the fulfillment of a condition of fact, the judge shall admit it upon, or subject to, the introduction of evidence sufficient to support a finding of the fulfillment of the condition.

Rule 615.
EXCLUSION OF WITNESSES

At the request of a party the judge shall order witnesses excluded so that they cannot hear the testimony of other witnesses, and he may make the order of his own motion. This rule does not authorize exclusion of (1) a party who is a natural person, or (2) an officer or employee of a party which is not a natural person designated as its representative by its attorney, or (3) a person whose presence is shown by a party to be essential to the presentation of his cause.

Rule 611.
MODE AND ORDER OF INTERROGATION AND PRESENTATION

(a) Control by Judge. The judge may exercise reasonable control over the mode and order of interrogating witnesses and presenting evidence so as to (1) make the interrogation and presentation effective for the ascertainment of the truth, (2) avoid needless consumption of time, and (3) protect witnesses from harassment or undue embarrassment.

(b) Scope of Cross-Examination. A witness may be cross-examined on any matter relevant to any issue in the case, including credibility. In the interests of justice, the judge may limit cross-examination with respect to matters not testified to on direct examination.

Rule 613.

PRIOR STATEMENTS OF WITNESSES

(a) Examining Witness Concerning Prior Statement. In examining a witness concerning a prior statement made by him, whether written or not, the statement need not be shown or its contents disclosed to him at that time, but on request the same shall be shown or disclosed to opposing counsel.

JUDICIAL NOTICE

Rule 201.

JUDICIAL NOTICE OF ADJUDICATIVE FACTS

(b) Kinds of Facts. A judicially noticed fact must be one not subject to reasonable dispute in that it is either (1) generally known within the territorial jurisdiction of the trial court or (2) capable of accurate and ready determination by resort to sources whose accuracy cannot reasonably be questioned.

(g) Instructing Jury. The judge shall instruct the jury to accept as established any facts judicially noticed.

RELEVANCE

Rule 401.

DEFINITION OF "RELEVANT EVIDENCE"

"Relevant evidence" means evidence having any tendency to make the existence of any fact that is of consequence to the determination of the action more probable or less probable than it would be without the evidence.

Rule 402.

RELEVANT EVIDENCE GENERALLY ADMISSIBLE; IRRELEVANT EVIDENCE INADMISSIBLE

All relevant evidence is admissible, except as otherwise provided by these rules, by other rules adopted by the Supreme Court, by Act of Congress, or by the Constitution of the United States. Evidence which is not relevant is not admissible.

COMPETENCY OF WITNESSES

Rule 601.

GENERAL RULE OF COMPETENCY

Every person is competent to be a witness except as otherwise provided in these rules.

Rule 602.

LACK OF PERSONAL KNOWLEDGE

A witness may not testify to a matter unless evidence is introduced sufficient to support a finding that he has personal knowledge of the matter. Evidence to prove personal knowledge may, but need not, consist of the testimony of the witness himself. This rule is subject to the provisions of Rule 703, relating to opinion testimony by expert witnesses.

EXPERT TESTIMONY

Rule 702.

TESTIMONY BY EXPERTS

If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise.

Rule 703.

BASES OF OPINION TESTIMONY BY EXPERTS

The facts or data in the particular case upon which an expert bases an opinion or inference may be those perceived by or made known to him at or before the hearing. If of a type reasonably relied upon by experts in the particular field in forming opinions or inferences upon the subject, the facts or data need not be admissible in evidence.

Rule 705.

DISCLOSURE OF FACTS OR DATA UNDERLYING EXPERT OPINION

The expert may testify in terms of opinion or inference and give his reasons therefore without prior disclosure of the underlying facts or data, unless the judge requires otherwise. The expert may in any event be required to disclose the underlying facts or data on cross-examination.

Rule 706.

COURT APPOINTED EXPERTS

(a) Appointment. The judge may on his own motion or on the motion of any party enter an order to show cause why expert witnesses should not be appointed, and may request the parties to submit nominations. The judge may appoint any expert witnesses agreed upon by the parties, and may appoint witnesses of his own selection. An expert witness shall not be appointed by the judge unless he consents to act. A witness so appointed shall be informed of his duties by the judge in writing, a copy of which shall be filed with the clerk, or at a conference in which the parties shall have opportunity to participate. A witness so appointed shall advise the parties of his findings, if any; his deposition may be taken by any party; and he may be called to testify by the judge or any party. He shall be subject to cross-examination by each party, including a party calling him as a witness.

HEARSAY

Rule 801.

DEFINITIONS

The following definitions apply under this Article:

(a) Statement. A "statement" is (1) an oral or written assertion or (2) nonverbal conduct of a person, if it is intended by him as an assertion.

(b) Declarant. A "declarant" is a person who makes a statement.

(c) Hearsay. "Hearsay" is a statement, other than one made by the declarant while testifying at the trial or hearing, offered in evidence to prove the truth of the matter asserted.

Rule 802.

HEARSAY RULE

Hearsay is not admissible except as provided by these rules or by other rules adopted by the Supreme Court or by Act of Congress.

Rule 803.

HEARSAY EXCEPTIONS: AVAILABILITY OF DECLARANT IMMATERIAL

The following are not excluded by the hearsay rule, even though the declarant is available as a witness:

(5) Recorded Recollection. A memorandum or record concerning a matter about which a witness once had knowledge but now has insufficient recollection to enable him to testify fully and accurately, shown to have been made when the matter was fresh in his memory and to reflect that knowledge correctly. If admitted, the memorandum or record may be read into evidence but may not itself be received as an exhibit unless offered by an adverse party.

(6) Records of Regularly Conducted Activity. A memorandum, report, record, or data compilation, in any form, of acts, events, conditions, opinions, or diagnoses, made at or near the time by, or from information transmitted by, a person with knowledge, all in the course of a regularly conducted activity, as shown by the testimony of the custodian or other qualified witness, unless the sources of information or other circumstances indicate lack of trustworthiness.

(18) Learned Treatises. To the extent called to the attention of an expert witness upon cross-examination or relied upon by him in direct examination, statements contained in published treatises, periodicals, or pamphlets on a subject of history, medicine, or other science or art, established as a reliable authority by the testimony or admission of the witness or by other expert testimony or by judicial notice. If admitted, the statements may be read into evidence but may not be received as exhibits.

IDENTIFICATION OF PERSONS AND SAMPLES

Rule 901.

REQUIREMENT OF AUTHENTICATION OR IDENTIFICATION

(a) General Provision. The requirement of authentication or identification as a condition precedent to admissibility is satisfied by evidence sufficient to support a finding that the matter in question is what its proponent claims.

(b) Illustrations. By way of illustration only, and not by way of limitation, the following are examples of authentication or identification conforming with the requirements of this rule:

(1) Testimony of Witness with Knowledge. Testimony that a matter is what it is claimed to be.

(3) Comparison by Trier or Expert Witness. Comparison by the trier of fact or by expert witnesses with specimens which have been authenticated.

(9) Process or System. Evidence describing a process or system used to produce a result and showing that the process or system produces accurate result.

ADMISSIBILITY AND PROOF OF SPECIAL MATTERS

Rule 406.

HABIT; ROUTINE PRACTICE

(a) Admissibility. Evidence of the habit of a person or of the routine practice of an organization, whether corroborated or not and regardless of the presence of eye-witnesses, is relevant to prove that the conduct of the person or organization on a particular occasion was in conformity with the habit or routine practice.

(b) Method of Proof. Habit or routine practice may be proved by testimony in the form of an opinion or by specific instances of conduct sufficient in number to warrant a finding that the habit existed or that the practice was routine.

Rule 612

WRITING USED TO REFRESH MEMORY

If a witness uses a writing to refresh his memory, either before or while testifying, an adverse party is entitled to have it produced at the hearing, to inspect it, to cross-examine the witness thereon, and to introduce in evidence those portions which relate to the testimony of the witness.

Rule 1006.

SUMMARIES

The contents of voluminous writings, recordings, or photographs which cannot conveniently be examined in court may be presented in the form of a chart, summary, or calculation. The originals, or duplicates, shall be made available for examination or copying, or both, by other parties at a reasonable time and place. The judge may order that they be produced in court.

KEY TO SELECTED GROUPS OF FRESHWATER ANIMALS

The following key is intended to provide an introduction to some of the more common freshwater animals. Technical language is kept to a minimum.

In using this key, start with the first couplet (1a, 1b), and select the alternative that seems most reasonable. If you selected "1a" you have identified the animal as a member of the group, Phylum PROTOZOA. If you selected "1b", proceed to the couplet indicated. Continue this process until the selected statement is terminated with the name of a group.

If you wish more information about the group, consult references. (See reference list.)

Key to Selected Groups of Freshwater Animals

1a The body of the organism comprising a single microscopic independent cell, or many similar and independently functioning cells associated in a colony with little or no difference between the cells i.e. without forming tissues; or body comprised of masses of multinucleate protoplasm. Mostly microscopic, single celled animals.

Phylum PROTOZOA

1b The body of the organism comprised of many cells of different kinds, i.e., forming tissues. May be microscopic or macroscopic. 2

2a Body or colony usually forming irregular masses or layers sometimes cylindrical, goblet shaped, vase shaped, or tree like. Size range from barely visible to large. 3

2b Body or colony shows some type of definite symmetry. 4

3a Colony surface rough or bristly in appearance under microscope or hand lens. Grey, green, or brown. Sponges.
Phylum PORIFERA (Fig. 1)

3b Colony surface relatively smooth. General texture of mass gelatinous, transparent. Clumps of minute individual organisms variously distributed. Moss animals, bryozoans.
Phylum BRYOZOA (Fig. 2)

4a Microscopic. Action of two ciliated (fringed) lobes at anterior (front) end in life often gives appearance of wheels. Body often segmented, accordian-like. Free swimming or attached. Rotifers or wheel animalcules.
Phylum TROCHELMINTHES (Rotifera) (Fig. 3)

4b Larger, wormlike, or having strong skeleton or shell. 5

5a Skeleton or shell present. Skeleton may be external or internal. 15

5b Body soft and/or wormlike. Skin may range from soft to parchment-like. 6

6a Three or more pairs of well formed jointed legs present. 19
Phylum ARTHROPODA (Fig. 4)

6b Legs or appendages, if present, limited to pairs of bumps or hooks. Lobes or tenacles, if present, soft and fleshy, not jointed. 7

7a Body strongly depressed or flattened in cross section. 8

7b Body oval, round, or shaped like an inverted "U" in cross section. 10

8a Parasitic inside bodies of higher animals. Extremely long and flat, divided into sections like a Roman girdle. Life history may involve an intermediate host. Tape worms.
Class CESTODA (Fig. 5)

8b Body a single unit. Mouth and digestive system present, but no anus. 9

9a External or internal parasite of higher animals. Sucking discs present for attachment. Life history may involve two or more intermediate hosts or stages. Flukes.
Class TREMATODA

9b Free living. Entire body covered with locomotive cilia. Eye areas in head often appear "crossed". Free living flatworms.
Class TURBELLARIA (Fig. 6)

10a Long, slender, with snake-like motion in life. Covered with glistening cuticle. Parasitic or free-living. Microscopic to six feet in length. Round worms.
Phylum NEMATHELMINTHES (Fig. 7)

10b Divided into sections or segments 11

10c Unsegmented. head blunt one or two retractile tentacles. Flat pointed, tail. 18

11a Head a more or less well-formed, hard, capsule with jaws, eyes, and antennae.
Class INSECTA order DIPTERA (Figs. 8A, 8C)

11b Head structure soft, except jaws (if present). Fig. 8E.) 12

12a Head conical or rounded, lateral appendages not conspicuous or numerous. 13

12b Head somewhat broad and blunt. Retractile jaws usually present. Soft fleshy lobes or tentacles, often somewhat flattened, may be present in the head region. Tail usually narrow. Lateral lobes or fleshy appendages on each segment unless there is a large sucker disc at rear end. 14
Phylum ANNELIDA (Fig. 9)

13a Minute dark colored retractile jaws present, body tapering somewhat at both ends, pairs or rings of bumps or "legs" often present, even near tail.
Class INSECTA Order DIPTERA (Fig. 8)

13b No jaws, sides of body generally parallel except at ends. Thickened area or ring usually present if not all the way back on body. Clumps of minute bristles on most segments. Earthworms, sludgeworms. 14
Order OLIGOCHAETA

14a Segments with bristles and/or fleshy lobes or other extensions. Tube builders, borers, or burrowers. Often reddish or greenish in color. Brackish or fresh water. Nereid worms.
Order POLYCHAETA (Fig. 9A)

14b Sucker disc at each end, the large one posterior. External blood-sucking parasites on higher animals, often found unattached to host. Leaches.
Class HIRUDINEA (Fig. 9B)

15a Skeleton internal, of true bone. (Vertebrates) 40

15b Body covered with an external skeleton or shell. 16
(Figs. 10, 13, 17, 18, 24, 25, 28)

16a External skeleton jointed, shell covers legs and other appendages, often leathery in nature. 19
Phylum ARTHROPODA

16b External shell entire, not jointed, unless composed of two clam-like halves. 17
(Figs. 10, 11, 12)

17a Half inch or less in length. Two leathery, clam-like shells. Soft parts inside include delicate jointed appendages. Phyllopods or branchiopods.
Class CRUSTACEA, Subclasses BRANCHIOPODA (Fig. 12) and OSTRACODA (Fig. 11)

17b Soft parts covered with thin skin, mucous produced, no jointed le 18
Phylum MOLLUSCA

18a Shell single, may be a spiral cone. Snails.
Class GASTROPODA (Fig. 13)

18b Shell double, two halves, hinged at one point. Mussels, clams.
Class BIVALVIA (Fig. 10)

19a Three pairs of regular walking legs, or their rudiments. Wings present in all adults and rudiments in some larvae. 29
Class INSECTA (Figs. 22, 24D 25, 26, 28, 29)

19b More than three pairs of legs apparently present. 20

20a Body elongated, head broad and flat

with strong jaws. Appendages following first three pairs of legs are rounded tapering filaments. Up to 3 inches long. Dobson fly and fish fly larvae.
Class INSECTA Order
MEGALOPTERA (Fig. 14)

20b Four or more pairs of legs. 21

21a Four pairs of legs. Body rounded, bulbous, head minute. Often brown or red. Water mites.

Phylum ARTHROPODA, Class
ARACHNIDA, Order ACARI
(Fig. 15)

21b Five or more pairs of walking or swimming legs; gills, two pairs of antennae. Crustaceans. 22
Phylum ARTHROPODA,
Class CRUSTACEA

22a Ten or more pairs of flattened, leaflike swimming and respiratory appendages. Many species swim constantly in life; some swim upside down. Fairy shrimps, phyllopods, or branchipods.
Subclass BRANCHIOPODA
(Fig. 16)

22b Less than ten pairs of swimming or respiratory appendages. 23

23a Body and legs inclosed in bivalved (2 halves) shell which may or may not completely hide them. 24

23b Body and legs not enclosed in bivalve shell. May be large or minute. 26
(Figs. 17, 18, 19)

24a One pair of branched antennae enlarged for locomotion, extend outside of shell (carapace). Single eye usually visible. "Water fleas"
Subclass CLADOCERA (Fig. 12)

24b Locomotion accomplished by body legs, not by antennae. 25

25a Appendages leaflike, flattened, more than ten pairs.
Subclass BRANCHIOPODA
(See 22 a)

25b Animal less than 3 mm, in length. Appendages more or less slender and jointed, often used for walking. Shells opaque. Ostracods.
(Fig. 11) Subclass OSTRACODA

26a Body a series of six or more similar segments, differing mainly in size. 27

26b Front part of body enlarged into a somewhat separate body unit (cephalothorax) often covered with a single piece of shell (carapace). Back part (abdomen) may be relatively small, even folded underneath front part. (Fig. 19b) 28

27a Body compressed laterally, i.e., organism is tall and thin. Scuds. amphipods.
Subclass AMPHIPODA (Fig. 17)

27b Body compressed dorsoventrally, i.e., organism low and broad. Flat gills contained in chamber beneath tail. Sowbugs.
Subclass ISOPODA (Fig. 18)

28a Abdomen extending straight out behind, ending in two small projections. One or two large masses of eggs are often attached to female. Locomotion by means of two enlarged, unbranched antennae, the only large appendages on the body. Copepods.
Subclass COPEPODA (Fig. 19)

28b Abdomen extending out behind ending in an expanded "flipper" or swimming paddle. Crayfish or craw fish. Eyes on movable stalks. Size range usually from one to six inches.
Subclass DECAPODA

29a Two pairs of functional wings, one pair may be more or less hardened as protection for the other pair. Adult insects which normally live on or in the water. (Figs. 25, 28) 39

29b No functional wings, though pads in which wings are developing may be visible. Some may resemble adult insects very closely, others may differ extremely from adults. 30

30a External pads or cases in which wings develop clearly visible.(Figs. 24, 26, 27) 35

30b More or less wormlike, or at least no external evidence of wing development. 31

31a No jointed legs present. Other structures such as hooks, sucker discs, breathing tubes may be present. Larvae of flies, midges, etc.
Order DIPTERA (Fig. 8)

31b Three pairs of jointed thoracic legs, head capsule well formed. 32

32a Minute (2-4mm) living on the water surface film. Tail a strong organ that can be hooked into a "catch" beneath the thorax. When released animal jumps into the air. No wings are ever grown. Adult springtails.
Order COLLEMBOLA (Fig. 20)

32b Larger (usually over 5 mm) wormlike, living beneath the surface. 33

33a Live in cases or webs in water. Cases or webs have a silk foundation to which tiny sticks, stones, and/or bits of debris are attached. Abdominal segments often with minute gill filaments. Generally cylindric in shape. Caddisfly larvae.
Order TRICHOPTERA (Fig. 21)

33b Free living, build no cases. 34

34a Somewhat flattened in cross section and massive in appearance. Each abdominal segment with rather stout, tapering, lateral filaments about as long as body is wide. Alderflies, fishflies, and dobsonflies.
Order MEGALOPTERA (Fig. 22, 14)

34b Generally rounded in cross section. Lateral filaments if present tend to be long and thin. A few forms extremely flattened, like a suction cup. Beetle larvae.
Order COLEOPTERA (Fig. 23)

35a Two or three filaments or other structures extending out from end of abdomen. 37

35b Abdomen ending abruptly, unless terminal segment itself is extended as single structure.(Figs. 24A, 24C) 36

36a Mouth parts adopted for chewing. Front of face covered by extensible folded mouthparts often called a "mask". Head broad, eyes widely spaced. Nymphs of dragonflies or darning needles.
Order ODONATA (Figs.24A, 24C, 24E)

36b Mouthparts for piercing and sucking. Legs often adapted for water locomotion. Body forms various. Water bugs, water scorpions, water boatmen, backswimmers, electric light bugs, water striders, water measurers, etc.
Order HEMIPTERA (Fig. 25)

37a Tail extensions (caudal filaments) two. Stonefly larvae.
Order PLECOPTERA (Fig. 26)

37b Tail extensions three, at times greatly reduced in size. 38

38a Tail extensions long and slender. Rows of hairs may give extensions a feather-like appearance. Mayfly larvae.
Order EPHEMEROPTERA (Fig. 27)

38b Tail extensions flat, elongated plates. Head broad with widely spaced eyes, abdomen relatively long and slender. Damselfly nymths.
Order ODONATA (Fig. 24D)

39a	External wings or wing covers form a hard protective dome over the inner wings folded beneath, and over the abdomen. Beetles. Order COLEOPTERA (Fig. 28)	
39b	External wings leathery at base. Membranaceous at tip. Wings sometimes very short. Mouthparts for piercing and sucking. Body form various. True bugs. Order HEMIPTERA (Fig. 25)	
40a	Appendage present in pairs. (fins, legs, wings).	42
40b	No paired appendages. Mouth a round suction disc.	41
41a	Body long and slender. Several holes along side of head. Lampreys. Sub Phylum VERTEBRATA, Class CYCLOSTOMATA	
41b	Body plump, oval. Tail extending out abruptly. Larvae of frogs and toads. Legs appear one at a time during metamorphosis to adult form. Tadpoles. Class AMPHIBIA	
42a	Paired appendages are legs	43
42b	Paired appendages are fins, gills covered by a flap (operculum). True fishes. Class PISCES	
43a	Digits with claws, nails, or hoofs	44
43b	Skin naked. No claws or digits Frogs, toads, and salamanders Class AMPHIBIA	
44a	Warm blooded	45
44b	Cold blooded. Body covered with horny scales or plates. Class REPTILIA	
45a	Body covered with feathers. Birds. Class AVES	
45b	Body covered with hair Mammals. Class MAMMALIA	

REFERENCES - Invertebrates

1 Eddy, Samuel and Hodson, A.C. Taxonomic Keys to the Common Animals of the North Central States. Burgess Pub. Company, Minneapolis. 162 p. 1961.

2 Edmondson, W.T. (ed.) and Ward and Whipple's Freshwater Biology. John Wiley & Sons, New York. pp. 1-1248. 1959.

3 Jahn, T.L. and Jahn, F.F. How to Know the Protozoa. Wm. C. Brown Company, Dubuque, Iowa. pp. 1-234. 1949.

4 Klots, Elsie B. The New Field Book of Freshwater Life. G.P. Putnam's Sons. 398 pp. 1966.

5 Kudo, R. Protozoology. Charles C. Thomas, Publisher, Springfield, Illinois. pp. 1-778. 1950.

6 Palmer, E. Lawrence. Fieldbook of Natural History. Whittlesey House, McGraw-Hill Book Company, Inc. New York. 1949.

7 Pennak, R.W. Freshwater Invertebrates of the United States. The Ronald Press Company, New York. pp. 1-769. 1953.

8 Pimentel, Richard A. Invertebrate Identification Manual. Reinhold Publishing Corp. 151 pp. 1967.

9 Pratt, H.W. A Manual of the Common Invertebrate Animals Exclusive of Insects. The Blaikston Company, Philadelphia, Pa. pp. 1-854. 1951.

REFERENCES - Fishes

1 American Fisheries Society. A List of Common and Scientific Names of Fishes from the United States and Canada. Special Publication No. 2, Am. Fish Soc. Executive Secretary AFS. Washington Bld. Suite 1040, 15th & New York Avenue, N.W. Washington, DC 20005. (Price $4.00 paper, $7.00 cloth). 1970.

2 Bailey, Reeve M. A Revised List of the Fishes of Iowa with Keys for Identification, IN: Iowa Fish and Fishing. State of Iowa, Super. of Printing. 1956. (Excellent color pictures).

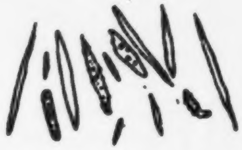
3 Eddy, Samuel. How to Know the Freshwater Fishes. Wm. C. Brown Company, Dubuque, Iowa. 1957.

4 Hubbs, C.L. and Lagler, K.F. Fishes of the Great Lakes Region. Bull. Cranbrook Inst. Science, Bloomfield Hills, Michigan. 1949.

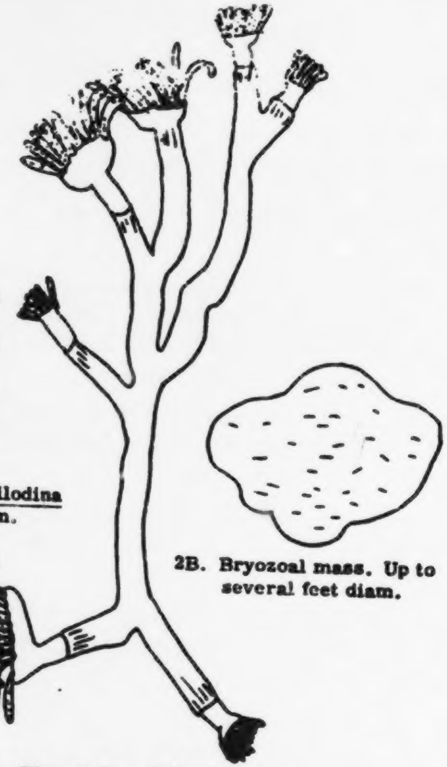
5 Lagler, K.F. Freshwater Fishery Biology. Wm. C. Brown Company, Dubuque, Iowa. 1952.

6 Trautman, M.B. The Fishes of Ohio. Ohio State University Press, Columbus. 1957. (An outstanding example of a State study).

Descriptors: Aquatic Life, Systematics.

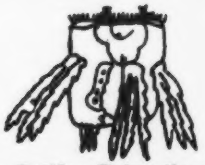


1. Spongilla spicules
Up to .2 mm. long.

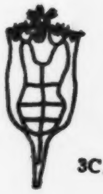


2A. Bryozoa, Plumatella. Individuals up to 2 mm. Intertwined masses may be very extensive.

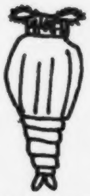
2B. Bryozoal mass. Up to several feet diam.



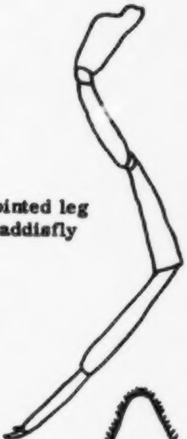
3A. Rotifer, Polyarthra
Up to .3 mm.



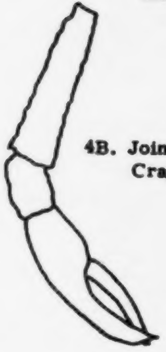
3B. Rotifer, Keratella
Up to .3 mm.



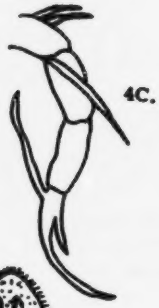
3C. Rotifer, Philodina
Up to .4 mm.



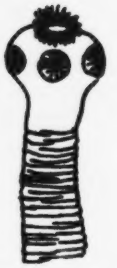
4A. Jointed leg
Caddisfly



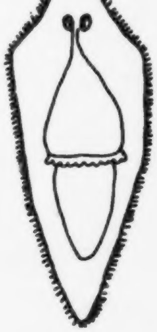
4B. Jointed leg
Crayfish



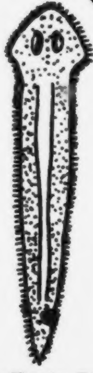
4C. Jointed leg
Ostracod



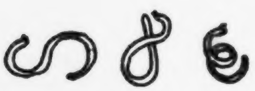
5. Tapeworm head,
Taenia. Up to
25 yds. long



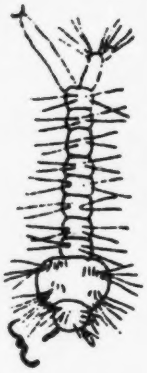
'aria, Mesostoma
to 1 cm.



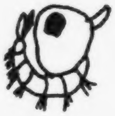
6B. Turbellaria, Dugesia
Up to 1.6 cm.



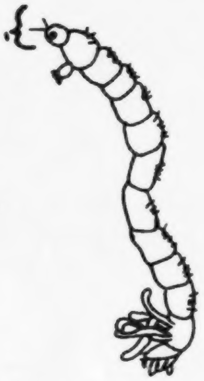
7. Nematodes. Free living
forms commonly up to
1 mm., occasionally
more.



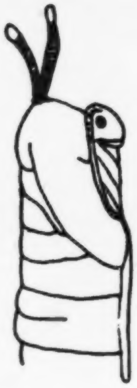
8A. Diptera, Mosquito larvae Up to 15 mm. long.



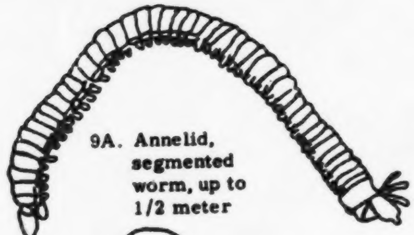
8B. Diptera, Mosquito pupa. Up to 5 mm.



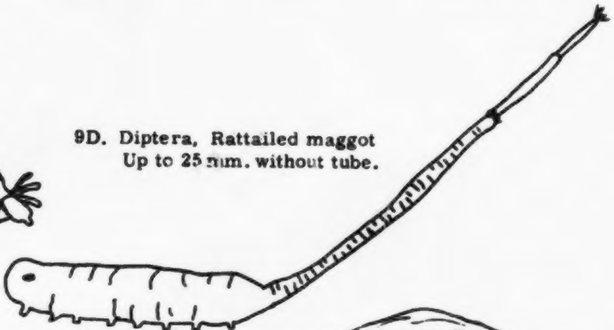
8C. Diptera, chironomid larvae. Up to 2 cm.



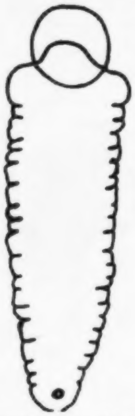
8E. Diptera, crane fly pupa. Up to 2.5 cm.



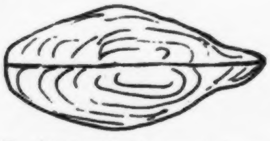
9A. Annelid, segmented worm, up to 1/2 meter



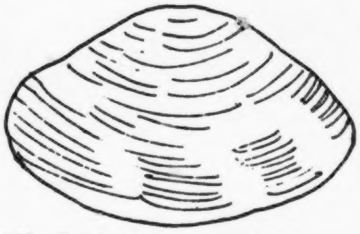
9D. Diptera, Rattailed maggot Up to 25 mm. without tube.



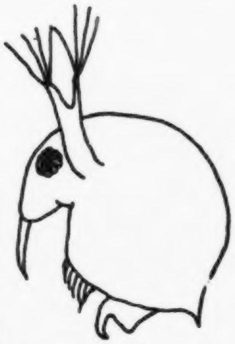
9B. Annelid, leech up to 20 cm.



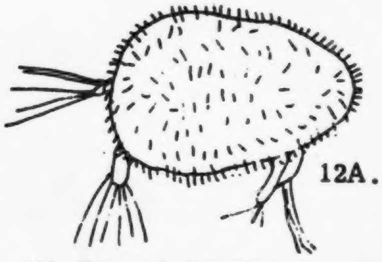
10B. Alasmidonta, end view.



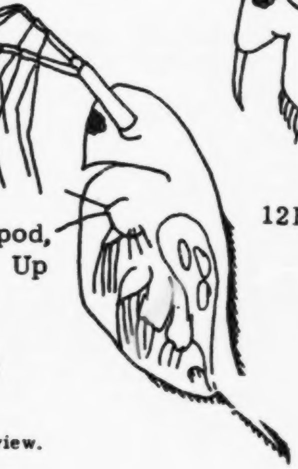
10A. Pelecyopod, Alasmidonta Side view, up to 18 cm. long.



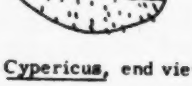
12B. Branchiopod, Bosmina. Up to 2mm.



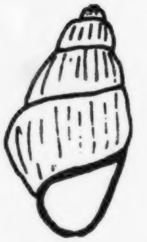
11A. Ostracod, Cypericus Side view, up to 7 mm.



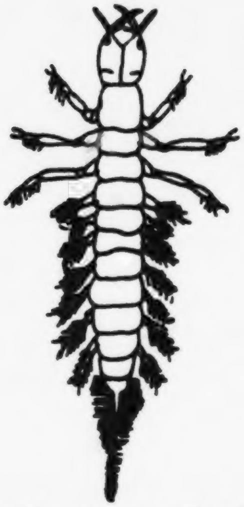
12A. Branchiopod, Daphnia. Up to 4mm.



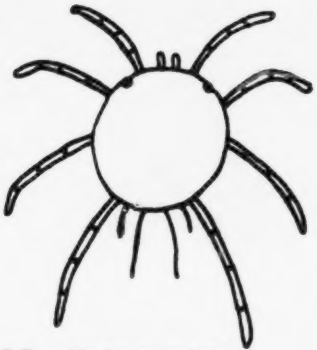
11B. Cypericus, end view.



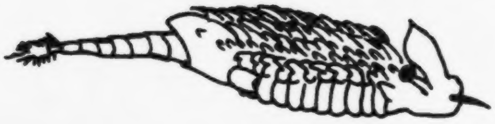
13. Gastropod, Campeloma
Up to 3 inches.



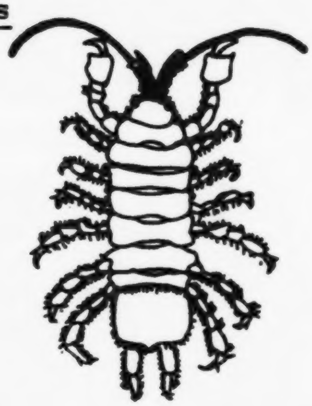
14. Megaloptera, Sialis
Alderfly larvae
Up to 25 mm.



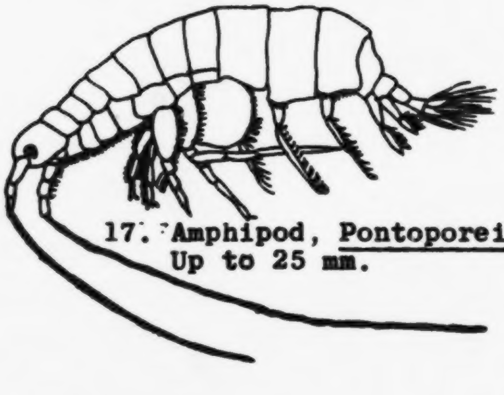
15. Water mite,
up to 3 mm.



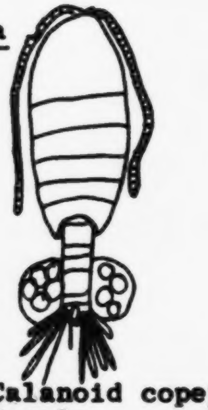
16. Fairy Shrimp, Eubranchipus
Up to 5 cm.



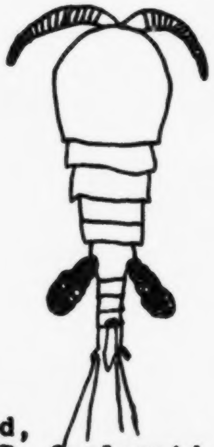
18. Isopod, Asellus
Up to 25 mm.



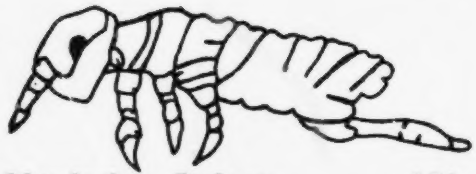
17. Amphipod, Pontoporeia
Up to 25 mm.



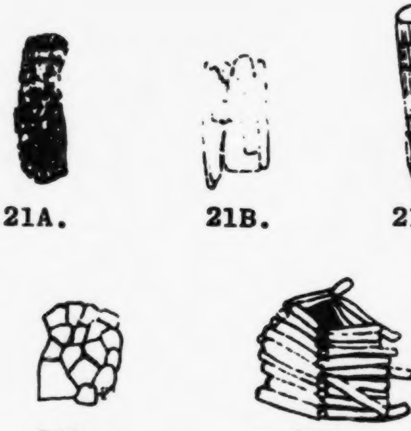
19A. Calanoid copepod,
Female
Up to 3 mm.



19B. Cyclopoid copepod
Female
Up to 25 mm.



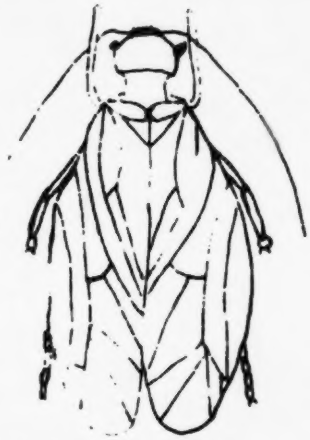
20. Collembola, Podura
Up to 2 mm. long



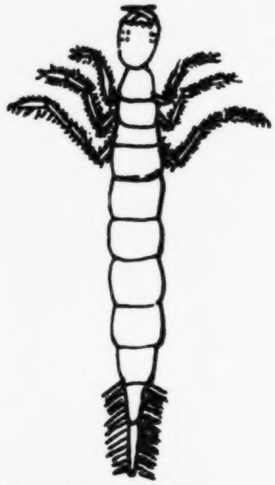
21A. 21B. 21C.

21D. 21E.

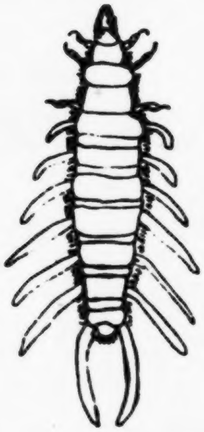
21. Trichoptera, larval cases, mostly 1-2 cm.



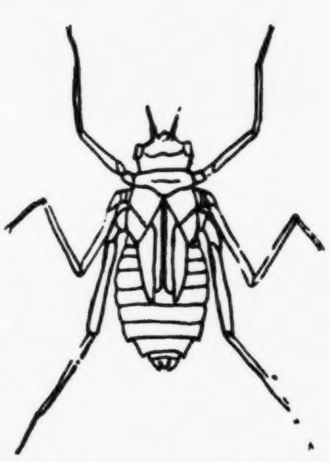
22. Megaloptera, alderfly
Up to 2 cm.



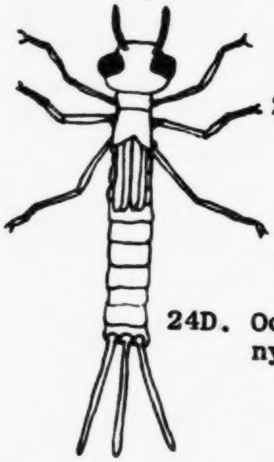
23A. Beetle larvae, Dytisidae, Usually about 2 cm.

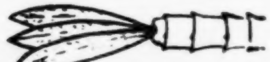


23B. Beetle larvae, Hydrophilidae Usually about 1 cm.



24A. Odonata, dragonfly nymph up to 3 or 4 cm

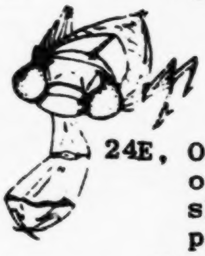




24B. Odonata, tail of damselfly nymph (side view)

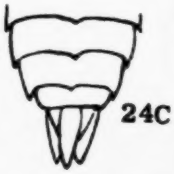
Suborder Zygoptera (24B, D)

24D. Odonata, damselfly nymph (top view)

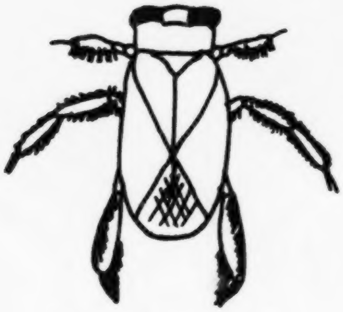


24E. Odonata, front view of dragonfly nymph showing "mask" partially extended

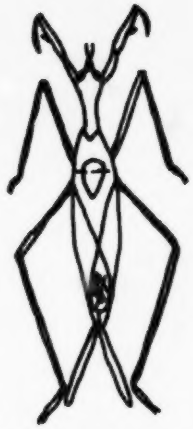
Suborder Anisoptera (24A, E, C)



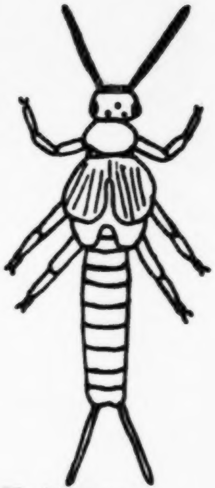
24C. Odonata, tail of dragonfly nymph (top view)



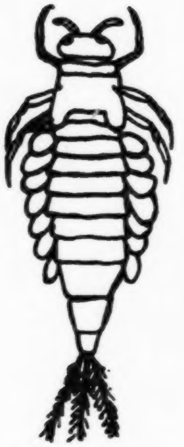
25A. Hemiptera,
Water Boatman
About 1 cm.



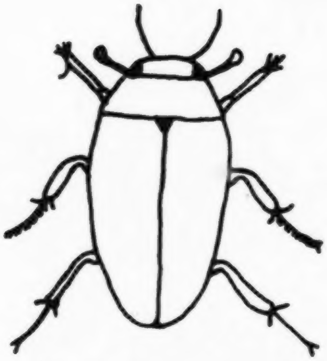
25B. Hemiptera,
Water Scorpion
About 4 cm.



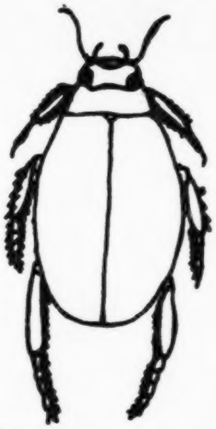
26. Plecoptera,
Stonefly nymph
Up to 5cm.



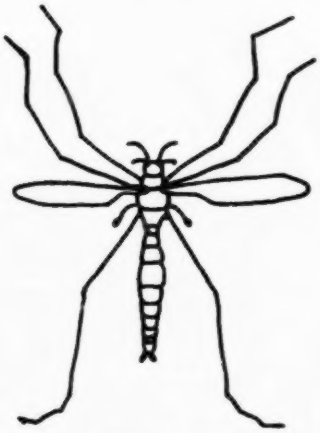
27. Ephemeroptera,
Mayfly nymph
Up to 3cm.



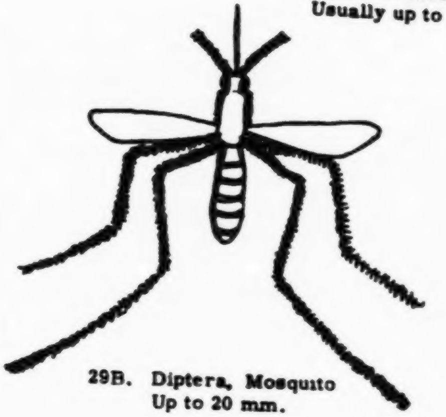
28A. Coleoptera,
Water scavenger
beetle. Up to 4 cm.



28B. Coleoptera,
Dytiscid beetle
Usually up to 4 cm.



29A. Diptera, Crane
fly. Up to 2½ cm.



29B. Diptera, Mosquito
Up to 20 mm.

II KEY TO ALGAE OF IMPORTANCE IN WATER POLLUTION

1	Plant a tube, thread, strand, ribbon, or membrane; frequently visible to the unaided eye	2
1'	Plants of microscopic cells which are isolated or in irregular, spherical, or microscopic clusters; cells not grouped into threads	123
2 (1)	Plant a tube, strand, ribbon, thread, or membrane composed of cells	3
2'	Plant a branching tube with continuous protoplasm, not divided into cells	120
3 (2)	Plant a tube, strand, ribbon, thread, or a mat of threads	4
3'	Plant a membrane of cells one cell thick (and 2 or more cells wide)	116
4 (3)	Cells in isolated or clustered threads or ribbons which are only one cell thick or wide	5
4'	Cells in a tube, strand, or thread all (or a part) of which is more than one cell thick or wide	108
5 (4)	Heterocysts present	6
5'	Heterocysts absent	23
6 (5)	Threads gradually narrowed to a point at one end	7
6'	Threads same width throughout	12
7 (6)	Threads as radii, in a gelatinous bead or mass	8
7'	Threads not in a gelatinous bead or mass	11
8 (7)	Spore (akinete) present, adjacent to the terminal heterocyst (Gloeotrichia)	9
8'	No spore (akinete) present (Rivularia)	10
9 (8)	Gelatinous colony a smooth bead	Gloeotrichia echinulata
9'	Gelatinous colony irregular	Gloeotrichia natans
10 (8')	Cells near the narrow end as long as wide	Rivularia dura
10'	Cells near the narrow end twice as long as wide	Rivularia haematites
11 (7')	Cells adjacent to heterocyst wider than heterocyst	Calothrix braunii
11'	Cells adjacent to heterocyst narrower than heterocyst	Calothrix parietina
12 (6')	Branching present	13
12'	Branching absent	14
13 (12)	Branches in pairs	Scytonema tolypothricoides
13'	Branches arising singly	Tolypothrix tenuis
14 (12')	Heterocyst terminal only (Cyclindrospermum)	15
14'	Hetrocysts intercalary (within the filament)	16
15 (14)	Heterocyst round	Cylindrospermum muscicola
15'	Heterocyst elongate	Cylindrospermum stagnale
16 (14')	Threads encased in a gelatinous bead or mass	17
16'	Threads not encased in a definite gelatinous mass	18
17 (16)	Heterocysts and vegetative cells rounded	Nostoc pruniforme
17'	Heterocysts and vegetative cells oblong	Nostoc carneum
18 (16')	Heterocysts and vegetative cells shorter than the thread width	Nodularia spumigena
18'	Heterocysts and vegetative cells not shorter than the thread width	19
19 (18')	Heterocysts rounded (Anabaena)	20
19'	Heterocysts clindric	Aphanizomenon flos-aquae
20 (19)	Cells elongate, depressed in the middle; heterocysts rare	Anabaena constricta
20'	Cells rounded; heterocysts common	21
21 (20')	Heterocysts with lateral extensions	Anabaena planctonica
21'	Heterocysts without lateral extensions	22

22 (21') Threads 4-8µ wide .... Anabaena flos-aquae
22' Threads 8-14µ wide .... Anabaena circinalis

23 (5') Branching absent 24
23' Branching (including "false" branching) present 84

24 (23) Cell pigments distributed throughout the protoplasm 25
24' Cell pigments limited to plastids 49

25 (23) Threads short and formed as an even spiral 285
25' Threads very long and not forming an even spiral 26

26 (25') Several parallel threads of cells in one common sheath .... Microcoleus subtorulosus
26' One thread per sheath if present 27

27 (26') Sheath or gelatinous matrix present 28
27' No sheath nor gelatinous matrix apparent (Oscillatoria) 35

28 (27) Sheath distinct; no gelatinous matrix between threads (Lyngbya) 29
28' Sheath indistinct or absent; threads interwoven with gelatinous matrix between (Phormidium) 32

29 (28) Cells rounded .... Lyngbya ocracea
29' Cells short cylindric 30

30 (29') Threads in part forming spirals .... Lyngbya lagerheimii
30' Threads straight or bent but not in spirals 31

31 (30') Maximum cell length 3.5µ ; sheath thin .... Lyngbya digueti
31' Maximum cell length 6.5µ ; sheath thick .... Lyngbya versicolor

32 (28') Ends of some threads with a rounded swollen "cap" cell 33
32' Ends of all threads without a "cap" cell 34

33 (32) End of thread (with "cap") abruptly bent .... Phormidium uncinatum
33' End of thread (with "cap") straight .... Phormidium autumnale

34 (32') Threads 3-5µ in width .... Phormidium inundatum
34' Threads 5-12µ in width .... Phormidium retzii

35 (27') Cells very short; generally less than 1/3 the thread diameter 36
35' Cells generally 1/2 as long to longer than the thread diameter 39

36 (35) Cross walls constricted .... Oscillatoria ornata
36' Cross walls not constricted 37

37 (36') Ends of thread, if mature, curved 38
37' Ends of thread straight .... Oscillatoria limosa

38 (37) Threads 10-14µ thick .... Oscillatoria curviceps
38' Threads 16-60µ thick .... Oscillatoria princeps

39 (35') Threads appearing red to purplish .... Oscillatoria rubescens
39' Threads yellow-green to blue-green 40

40 (39') Threads yellow-green 41
40' Threads blue-green 43

41 (40) Cells 4-7 times as long as the thread diameter .... Oscillatoria putrida
41' Cells less than 4 times as long as the thread diameter 42

42 (41') Prominent granules ("pseudovacuoles") in center of each cell .... Oscillatoria lauterbornii
42' No prominent granules in center of cells .... Oscillatoria chlorina

43 (40') Cells 1/2-2 times as long as the thread diameter 44
43' Cells 2-3 times as long as the thread diameter 48

44 (43) Cell walls between cells thick and transparent .... Oscillatoria pseudogeminata
44' Cell walls thin, appearing as a dark line 45

45 (44') Ends of thread straight .... *Oscillatoria agardhii*
45' Ends of mature threads curved 46

46 (45') Prominent granules present especially at both ends of each cell .... *Oscillatoria tenuis*
46' Cells without prominent granules 47

47 (46') Cross walls constricted .... *Oscillatoria chalybea*
47' Cross walls not constricted .... *Oscillatoria formosa*

48 (43') End of thread long tapering .... *Oscillatoria splendida*
48' End of thread not tapering .... *Oscillatoria amphibia*

49 (24') Cells separate from one another and enclosed in a tube (*Cymbella*) .... 251'
49' Cells attached to one another as a thread or ribbon 50

50 (49') Cells separating readily into discs or short cylinders, their circular face showing radial markings 233
50' Cells either not separating readily, or if so, no circular end wall with radial markings 51

51 (50') Cells in a ribbon, attached side by side or by their corners 52
51' Cells in a thread, attached end to end 56

52 (51) Numerous regularly spaced markings in the cell wall 53
52' Numerous markings in the cell wall absent (*Scenedesmus*) 128

53 (52) Wall markings of two types, one coarse, one fine 185
53' Wall markings all fine (*Fragilaria*) 54

54 (53') Cells attached at middle portion only .... *Fragilaria crotonensis*
54' Cells attached along entire length 55

55 (54') Cell length 25-100μ .... *Fragilaria capucina*
55' Cell length 7-25μ .... *Fragilaria construens*

56 (51') Plastid in the form of a spiral band (*Spirogyra*) 57
56' Plastid not a spiral band 61

57 (56) One plastid per cell 58
57' Two or more plastids per cell 60

58 (57) Threads 18-26μ wide .... *Spirogyra communis*
58' Threads 28-50μ wide 59

59 (58') Threads 28-40μ wide .... *Spirogyra varians*
59' Threads 40-50μ wide .... *Spirogyra porticalis*

60 (57') Threads 30-45μ wide; 3-4 plastids per cell .... *Spirogyra fluviatilis*
60' Threads 50-80μ wide; 5-8 plastids per cell .... *Spirogyra majuscula*

61 (56') Plastids two per cell 62
61' Plastids either one or more than two per cell 66

62 (61) Cells with knobs or granules on the wall 63
62' Cells with a smooth outer wall 64

63 (62) Each cell with two central knobs on the wall .... *Desmidium grevillii*
63' Each cell with a ring of granules near one end .... *Hyalotheca mucosa*

64(62') Cells dense green, each plastid reaching to the wall .... *Zygnema sterile*
64' Cells light green, plastids not completely filling the cell 65

65 (64') Width of thread 26-32μ; maximum cell length 60μ .... *Zygnema insigne*
65' Width of thread 30-36μ; maximum cell length 120μ .... *Zygnema pectinatum*

66 (61') Plastid a wide ribbon, passing through the cell axis (*Mougeotia*) 67
66' Plastid or plastids close to the cell wall (parietal) 69

67 (66)	Threads with occasional "knee-joint" bends	Mougeotia genuflexa
67'	Threads straight	68
68 (67')	Threads 19-24μ wide; pyrenoids 4-16 per cell	Mougeotia sphaerocarpa
68'	Threads 20-34μ wide; pyrenoids 4-10 per cell	Mougeotia scalaris
69 (66')	Occasional cells with one to several transverse wall lines near one end (Oedogonium)	70
69'	Occasional terminal transverse wall lines not present	73
70 (69)	Thread diameter less than 24μ	71
70'	Thread diameter 25μ or more	72
71 (70)	Thread diameter 9-14μ	Oedogonium suecicum
71'	Thread diameter 14-23μ	Oedogonium boscii
72 (70)	Dwarf male plants attached to normal thread, when reproducing	Oedogonium idioandrosporum
72'	No dwarf male plants produced	Oedogonium grande
73 (69')	Cells with one plastid which has a smooth surface	74
73'	Cells with several plastids or with one nodular plastid	78
74 (73)	Cells with rounded ends	Stichococcus bacillaris
74'	Cells with flat ends (Ulothrix)	75
75 (74')	Threads 10μ or less in diameter	76
75'	Threads more than 10μ in diameter	77
76 (75)	Threads 5-6μ in diameter	Ulothrix variabilis
76'	Threads 6-10μ in diameter	Ulothrix tenerrima
77 (75')	Threads 11-17μ in diameter	Ulothrix aequalis
77'	Threads 20-60μ in diameter	Ulothrix Zonata
78 (73')	Iodine test for starch positive; one nodular plastid per cell	79
78'	Iodine test for starch negative; several plastids per cell	80
79 (78)	Thread when broken, forming "H" shape segments	Microspora amoena
79'	Thread when fragmented, separating irregularly or between cells (Rhizoclonium)	100
80 (78')	Side walls of cells straight, not bulging. A pattern of fine lines or dots present in the wall but often indistinct (Melosira)	81
80'	Side walls of cells slightly bulging. Pattern of wall markings not present (Tribonema)	83
81 (80)	Spine-like teeth at margin of end walls	82
81'	No spine-like teeth present	Melosira varians
82 (81)	Wall with fine granules, arranged obliquely	Melosira crenulata
82'	Wall with coarse granules, arranged parallel to sides	Melosira granulata
83 (80')	Plastids 2-4 per cell	Tribonema minus
83'	Plastids more than 4 per cell	Tribonema bombycinum
84 (23')	Plastids present; branching "true"	85
84'	Plastids absent; branching "false"	Plectonema tomasiniana
85 (84)	Branches reconnected, forming a net	Hydrodictyon reticulatum
85'	Branches not forming a distinct net	86
86 (85')	Each cell in a conical sheath open at the broad end (Dinobryon)	87
86'	No conical sheath around each cell	90
87 (86)	Branches diverging, often almost at a right angle	Dinobryon divergens
87'	Branches compace often almost parallel	88
88 (87')	Narrow end of sheath sharp pointed	89
88'	Narrow end of sheath blunt pointed	Dinobryon sertularia

89 (88)	Narrow end drawn out into a stalk	*Dinobryon stipitatum*
89'	Narrow end diverging at the base	*Dinobryon sociale*
90 (86')	Short branches on the main thread in whorls of 4 or more (Nitella)	91
90'	Branching commonly single or in pairs	92
91 (90)	Short branches on the main thread rebranched once	*Nitella flexilis*
91'	Short branches on the main thread rebranched two to four times	*Nitella gracilis*
92 (90')	Terminal cell each with a colorless spine having an abruptly swollen base (*Bulbochaete*)	93
92'	No terminal spines with abruptly swollen bases	94
93	Vegetative cells 20-48μ long	*Bulbochaete mirabilis*
93'	Vegetative cells 48-88μ long	*Bulbochaete insignis*
94 (92')	Cells red, brown, or violet	*Audouinella violacea*
94'	Cells green	95
95 (94')	Threads enclosed in a gelatinous bead or mass	96
95'	Threads not surrounded by a gelatinous mass	99
96 (95)	Abrupt change in width from main thread to branches (*Draparnaldia*)	97
96'	Gradual change in width from main thread to branches (*Chaetophora*)	98
97 (96)	Branches (from the main thread) with a central, main axis	*Draparnaldia plumosa*
97'	Branches diverging and with no central main axis	*Draparnaldia glomerata*
98 (96')	End cells long-pointed, with colorless tips	*Chaetophora attenuata*
98'	End cells abruptly pointed, mostly without long colorless tips	*Chaetophora elegans*
99 (95')	Light and dense dark cells intermingled in the thread	*Pithophora oedogogonia*
99'	Most of the cells essentially alike in density	100
100 (99')	Branches few in number, and short, colorless	*Rhizoclonium hieroglyphicum*
100'	Branches numerous and green	101
101 (100')	Terminal attenuation gradual, involving two or more cells (*Stigeoclonium*)	102
101'	Terminal attenuation absent or abrupt, involving only one cell (*Cladophora*)	104
102 (101)	Branches frequently in pairs	103
102'	Branches mostly single	*Stigeoclonium stagnatile*
103 (102)	Cells in main thread 1-2 times as long as wide	*Stigeoclonium lubricum*
103'	Cells in main thread 2-3 times as long as wide	*Stigeoclonium tenue*
104 (101')	Branching often appearing forked, or in threes	*Cladophora aegagropila*
104'	Branches distinctly lateral	105
105 (104')	Branches forming acute angle with main thread, thus forming clusters	*Cladophora glomerata*
105'	Branches forming wide angles with the main thread	106
106 (105')	Threads crooked and bent	*Cladophora fracta*
106'	Threads straight	107
107 (106')	Branches few, seldom rebranching	*Cladophora insignis*
107'	Branches numerous, often rebranching	*Cladophora crispata*
108 (4')	Plant or tube with a tight surface layer of cells and with regularly spaced swellings (nodes).	*Lemanea annulata*
108'	Plant not a tube that has both a tight layer of surface cells and nodes	109
109 (108')	Cells spherical and loosely arranged in a gelatinous matrix	*Tetraspora gelatinosa*
109'	Cells not as loosely arranged spheres	110
110 (109')	Plants branch	111
110'	Plants not branched	*Schizomeris leibleinii*
111 (110)	Clustered branching	112
111'	Branches single	115

112 (111) Threads embedded in gelatinous matrix (Batrachospermum)........ 113
112' No gelantinous matrix (Chara)........ 114

113 (112) Nodal masses of branches touching one another........ Batrachospermum vagum
113' Nodal masses of branches separated by a narrow space..... Batrachospermum moniliforme

114 (112') Short branches with 2 naked cells at the tip........ Chara globularis
114' Short branches with 3-4 naked cells at the tip........ Chara vulgaris

115 (111') Heterocysts present; plastids absent........ Stigonema minutum
115' Heterocysts absent; plastids present........ Compsopogon coeruleus

116 (3') Red eye spot and two flagella present for each cell........ 125
116' No eye spots nor flagella present........ 117

117 (116') Round to oval cells, held together by a flat gelatinous matrix (Agmenellum)........ 131
117' Cells not round and not enclosed in a gelatinous matrix........ 118

118 (117') Cells regularly arranged to an unattached disc. Number of cells 2, 4, 8, 16, 32, 64, or 128........ 133'
118' Cells numerous; membrane attached on one surface........ 119

119 (118') Long hairs extending from upper surface of cells........ Chaetopeltis megalocystis
119' No hairs extending from cell surfaces........ Hildenbrandia rivularis

120 (2') Constriction at the base of every branch........ Dichotomosiphon tuberosus
120' No constrictions present in the tube (Vaucheria)........ 121

121 (120') Egg sac attached directly, without a stalk, to the main vegetative tube... Vaucheria sessilis
121' Egg sac attached to an abrupt, short, side branch........ 122

122 (121') One egg sac per branch........ Vaucheria terrestris
122' Two or more egg sacs per branch........ Vaucheria geminata

123 (1') Cells in colonies generally of a definite form or arrangement........ 124
123' Cells isolated, in pairs or in loose, irregular aggregates........ 173

124 (123) Cells with many transverse rows of markings on the wall........ 185
124' Cells without transverse rows of markings........ 125

125 (124') Cells arranged as a layer one cell thick........ 126
125' Cell cluster more than one cell thick and not a flat plate........ 137

126 (125) Red eye spot and two flagella present for each cell........ Gonium pectorale
126' No red eye spots nor flagella present........ 127

127 (126') Cells elongate, united side by side in 1 or 2 rows (Scenedesmus)........ 128
127' Cells about as long as wide........ 131

128 (127) Middle cells without spines but with pointed ends........ Scenedesmus dimorphus
128' Middle cells with rounded ends........ 129

129 (128'') Terminal cells with spines........ 130
129' Terminal cells without spines........ Scenedesmus bijuga

130 (129) Terminal cells with two spines each........ Scenedesmus quadricauda
130' Terminal cells with three or more spines each........ Scenedesmus abundans

131 (117) Cells in regular rows, immersed in colorless matrix (Agmenellum quadriduplicatum)... 132
131' Cells not immersed in colorless matrix........ 133

132 (131) Cell diameter 1.3 to 2.2μ........ Agmenellum quadriduplicatum, tenuissima type
132' Cell diameter 3-5μ........ Agmenellum quadriduplicatum, glauca type

133 (131') Cells without spines, projections, or incisions........ Crucigenia quadrata
133' Cells with spines, projections, or incisions........ 134

134 (133')	Cells rounded	*Micractinium pusillum*
134'	Cells angular (*Pediastrum*)	135
135 (134')	Numerous spaces between cells	*Pediastrum duplex*
135'	Cells fitted tightly together	136
136 (135')	Cell incisions deep and narrow	*Pediastrum tetras*
136'	Cell incisions shallow and wide	*Pediastrum boryanum*
137 (125')	Cells sharp-pointed at both ends; often arcuate	138
137'	Cells not sharp-pointed at both ends; not arcuate	141
138 (137)	Cells embedded in a gelatinous matrix	*Kirchneriella lunaris*
138'	Cells not embedded in a gelatinous matrix	139
139 (138')	Cells all arcuate; arranged back to back	*Selenastrum gracile*
139'	Cells straight or bent in various ways; loosely arranged or twisted together (*Ankistrodesmus*)	140
140 (139')	Cells bent	*Ankistrodesmus falcatus*
140'	Cells straight	*Ankistrodesmus falcatus* var. *acicularis*
141 (137')	Flagella present; eye spots often present	142
141'	No flagella nor eye spots present	152
142 (141)	Each cell in a conical sheath open at the wide end (*Dinobryon*)	86
142'	Individual cells not in conical sheaths	143
143 (142')	Each cell with 1-2 long straight rods extending	*Chrysosphaerella longispina*
143'	No long straight rods extending from the cells	144
144 (143')	Cells touching one another in a dense colony	145
144'	Cells embedded separately in a colorless matrix	149
145 (144)	Cells arranged radially, facing outward	146
145'	Cells all facing in one direction	147
146 (145)	Plastids brown; eye spot absent	*Synura uvella*
146'	Plastids green; eye spot present in each cell	*Pandorina morum*
147 (145')	Each cell with 4 flagella	*Spondylomorum quaternarium*
147'	Each cell with 2 flagella (*Pyrobotrys*)	148
148 (147')	Eye spot in the wider (anterior) end of the cell	*Pyrobotrys stellata*
148'	Eye spot in the narrower (posterior) end of the cell	*Pyrobotrys gracilis*
149 (144')	Plastids brown	*Uroglenopsis americana*
149'	Plastids green	150
150 (149')	Cells 16, 32, or 64 per colony	*Eudorina elegans*
150'	Cells more than 100 per colony	151
151 (150')	Colony spherical; each cell with an eye spot	*Volvox aureus*
151'	Colony tubular or irregular; no eye spots (*Tetraspora*)	109
152 (141')	Elongate cells, attached together at one end; arranged radially (*Actinastrum*)	153
152'	Cells not elongate, often spherical	154
153 (152)	Cells cylindric	*Actinastrum gracillimum*
153'	Cells distinctly bulging	*Actinastrum hantzschii*
154 (152')	Plastids present	155
154'	Plastids absent; pigment throughout each protoplast	168
155 (154)	Colonies, including the outer matrix, orange to red-brown	*Botryococcus braunii*
155'	Matrix, if any, not bright colored; cell plastids green	156

156 (155')	Colonies round to oval	160
156'	Colonies not round, often irregular in form	157
157 (156')	Straight (flat) walls between adjacent cells (Phytoconis)	278
157'	Walls between neighboring cells rounded	158
158 (157')	Cells arranged as a surface layer in a large gelatinous tube (Tetraspora)	109
158'	Colony not a tube; cells in irregular pattern	159
159 (158')	Large cells more than twice the diameter of the small cells (Chlorococcum)	280'
159'	Large cells not more than twice the diameter of the small cells (Palmella)	281
160 (156)	Cells touching one another; tightly grouped	Coelastrum microporum
160'	Cells loosely grouped	161
161 (160')	Colorless threads extend from center of colony to cells	162
161'	No colorless threads attached to cells in colony	164
162 (161)	Cells rounded or straight, oval (Dictyosphaerium)	163
162'	Cells elongate, some cells curved	Dimorphococcus lunatus
163 (162)	Cells rounded	Dictyosphaerium pulchellum
163'	Cells straight, oval	Dictyosphaerium ehrenbergianum
164 (161')	Cells rounded	165
164'	Cells oval	Oocystis borgei
165 (164)	One plastid per cell	166
165'	Two to four plastids per cell	Gloeococcus schroeteri
166 (165)	Outer matrix divided into layers (Gloeocystis)	167
166'	Outer matrix homogeneous	Sphaerocystis schroeteri
167 (166)	Colonies angular	Gloeocystis planctonica
167'	Colonies rounded	Gloeocystis gigas
168 (154')	Cells equidistant from center of colony (Gomphosphaeria)	169
168'	Cells irregularly distributed in the colony	172
169 (168)	Cells with pseudovacuoles	Gomphospaeria wichurae
169'	Cells without pseudovacuoles	170
170 (169')	Cells 2-4μ in diameter (Gomphosphaeria lacustris)	171
170'	Cells ovate	Gomphosphaeria aponina
171 (170)	Cells spherical	Gomphosphaeria lacustris, kuetzingianum type
171'	Cells 4-15 in diameter	Gomphosphaeria lacustris, collinsii type
172 (168')	Cells ovid; division plane perpendicular to long axis (Coccochloris)	286
172'	Cells rounded; or division plane perpendicular to short axis (Anacystis)	286'
173 (123')	Cells with an abrupt median transverse groove or incision	174
173'	Cells without an abrupt transverse median groove or incision	184
174 (173)	Cells brown; flagella present (armored flagellates)	175
174'	Cells green; no flagella (desmids)	178
175 (174)	Cell with 3 or more long horns	Ceratium hirundinella
175'	Cell without more than 2 horns	176
176 (175')	Cell wall of very thin smooth plates	Glenodinium palustre
176'	Cell wall of very thick rough plates (Peridinium)	177
177 (176')	Ends of cell pointed	Peridinium wisconsinense
177'	Ends of cell rounded	Peridinium cinctum
178 (174')	Margin of cell with sh[illegible] pointed, deeply cut lobes or long spikes	179
178'	Lobes, if present, with rounded ends	182

179 (178) Median incision narrow, linear .... *Micrasterias truncata*
179' Median incision wide, "V" or "U" shaped (*Staurastrum*) 180

180 (179) Margin of cell with long spikes .... *Staurastrum paradoxum*
180' Margin of cell without long spikes 181

181 (180") Ends of lobes with short spines .... *Staurastrum polymorphum*
181' Ends of lobes without spines .... *Staurastrum punctulatum*

182 (178') Length of cell about double the width .... *Euastrum oblongum*
182' Length of cell one to one and one-half times the width (*Cosmarium*) 183

183 (182') Median incision narrow linear .... *Cosmarium botrytis*
183' Median incision wide, "U" shaped .... *Cosmarium portianum*

184 (173') Cells triangular .... *Tetraedron muticum*
184' Cells not triangular 185

185 (124) Cells with one end distinctly different from the other 186
185' Cells with both ends essentially alike 225

186 (185) Numerous transverse (not spiral) regularly spaced wall markings present (diatoms) 187
186' No transverse regularly spaced wall markings 193

187 (186) Cells curved (bent) in girdle view .... *Rhoicosphenia curvata*
187' Cells not curved in girdle view 188

188 (187') Cells with both fine and coarse transverse lines .... *Meridion circulare*
188' Cells with transverse lines all alike in thickness 189

189 (188') Cells essentially linear to rectangular; one terminal swelling larger than the other .... (*Asterionella*) 190
189' Cells wedge-shaped; margins sometimes wavy (*Gomphonema*) 191

190 (189) Larger terminal swelling 1-1/2 to 2 times wider than the other .... *Asterionella formosa*
190' Larger terminal swelling less than 1-1/2 times wider than the other .. *Asterionella gracillima*

191 (189') Narrow end enlarged in valve view .... *Gomphonema geminatum*
191' Narrow end not enlarged in valve view 192

192 (191') Tip of broad end about as wide as tip of narrow end in valve view .... *Gomphonema parvulum*
192' Tip of broad end much wider than tip of narrow end in valve view .. *Gomphonema olivaceum*

193 (186') Spine present at each end of cell .... *Schroederia setigera*
193' No spine on both ends of cell 194

194 (193') Pigments in one or more plastids 195
194' No plastid; pigments throughout the protoplast .... *Entophysalis lemaniae*

195 (194) Cells in a conical sheath (*Dinobryon*) 86
195' Cells not in a conical sheath 196

196 (195') Cell covered with scales and long spines .... *Mallomonas caudata*
196' Cells not covered with scales and long spines 197

197 (196') Protoplasts separated by a space from a rigid sheath (lorica) 198
197' No loose sheath around the cells 202

198 (197) Cells compressed (flattened) .... *Phacotus lenticularis*
198' Cells not compressed 199

199 (198') Lorica opaque; yellow to reddish or brown .... *Trachelomonas crebea*
199' Lorica transparent; colorless to brownish (*Chrysococcus*) 200

200 (199') Outer membrane (lorica) oval .... *Chrysococcus ovalis*
200' Outer membrane (lorica) rounded 201

201 (200') Lorica thickened around opening .... *Chrysococcus rufescens*
201' Lorica not thickened around opening .... *Chrysococcus major*

202 (197') Front end flattened diagonally 203
202' Front end not flattened diagonally 206

203 (202) Plastids bright blue-green (*Chroomonas*) 204
203' Plastids brown, red, olive-green, or yellowish 205

204 (203) Cell pointed at one end .... *Chroomonas nordstetii*
204' Cell not pointed at one end .... *Chroomonas setoniensis*

205 (203') Gullet present; furrow absent .... *Cryptomonas erosa*
205' Furrow present; gullet absent .... *Rhodomonas lacustris*

206 (202') Plastids yellow-brown .... *Chromulina rosanoffi*
206' Plastids not yellow-brown; generally green 207

207 (206') One plastid per cell 208
207' Two to several plastids per cell 211

208 (207) Cells tapering at each end .... *Chlorogonium euchlorum*
208' Cells rounded to oval 209

209 (208') Two flagella per cell (*Chlamydomonas*) 210
209' Four flagella per cell .... *Cateria multifilis*

210 (209) Pyrenoid angular; eye spot in front third of cell .... *Chlamydomonas reinhardi*
210' Pyrenoid circular; eye spot in middle third of cell .... *Chlamydomonas globosa*

211 (207') Two plastids per cell .... *Cryptoglena pigra*
211' Several plastids per cell 212

212 (211') Cell compressed (flattened) (*Phacus*) 213
212' Cell not compressed 214

213 (212) Posterior spine short, bent .... *Phacus pleuronectes*
213' Posterior spine long, straight .... *Phacus longicauda*

214 (212) Cell margin rigid 215
214' Cell margin flexible (*Euglena*) 217

215 (214) Cell margin with spiral ridges .... *Phacus pyrum*
215' Cell margin without ridges, but may have spiral lines (*Lepocinclis*) 216

216 (215') Posterior end with an abrupt, spine-like tip .... *Lepocinclis ovum*
216' Posterior end rounded .... *Lepocinclis texta*

217 (214') Green plastids hidden by a red pigment in the cell .... *Euglena sanguinea*
217' No red pigment except for the eye spot 218

218 (217') Plastids at least 1/4 the length of the cell 219
218' Plastids discoid or at least shorter than 1/4 the length of the cell 220

219 (218) Plastids two per cell .... *Euglena agilis*
219' Plastids several per cell, often extending radiately from the center .... *Euglena viridis*

220 (218') Posterior end extending as an abrupt colorless spine 221
220' Posterior end rounded or at least with no colorless spine 222

221 (220) Spiral markings very prominent and granular .... *Euglena spirogyra*
221' Spiral markings fairly prominent, not granular .... *Euglena oxyuris*

222 (220') Small; length 35-55μ .... *Euglena gracilis*
222' Medium to large; length 65μ or more 223

223 (222') Medium in size; length 65-200μ 224
223' Large in size; length 250-290μ .... *Euglena ehrenbergii*

224 (223) Plastids with irregular edge; flagellum 2 times as long as cell.........*Euglena polymorpha*
224' Plastids with smooth edge; flagellum about 1/2 the length of the cell.........*Euglena deses*

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227' Cell long and narrow; width up to 5µ.........*Closterium aciculare*

228 (226') Cell with a narrow abrupt spine at each blunt end.........*Ophiocytium capitatum*
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237' Outer valve zone constituting more than 1/2 the diameter.........*Cyclotella compta*

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239' Cell without two transverse bands, in girdle view.........*Stephanodiscus hantzschii*

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263' Transverse lines (striae) present across transverse axis of valve face 264

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275 (274') Cells up to 65 times as long as wide; central area absent to small oval .... Synedra acus var. radians
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283' Cell 3-5μ in diameter; pyrenoid distinct .... Chlorella pyrenoidosa

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285' Thread non-septate (without crosswalls) .... Spirulina nordstedtii

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289 (288') Cell 6-12μ in diameter; cells in colonies are mostly spherical.......... *Anacystis thermalis*
289' Cell 12-50μ in diameter; cells in colonies are often angular........... *Anacystis dimidiata*

FOREWORD

The following work is more easily defined in terms of what it is not, than what it is; it is not a "key" in the usual biological sense of the word, nor is it a glossary of a dictionary. It is rather a device for determining what general kind of organism or group is designated by some unrecognized name, be it common or scientific.

If one has access to one or more of the references cited, he can find the same name, and learn much more about it; but not everyone has all of these books, and the information is often couched in highly technical terms.

The nonbiologist would be well advised to read Part I before attempting to use the Finder. The experienced biologist on the other hand may proceed directly to the index and quickly be referred to the larger group to which his unknown organism belongs.

No professional systematist will find himself completely at home. In an effort to present a relatively simple concept of relationships couched in standard terms for all groups or organisms, some violence was done to certain highly sophisticated systems of classification. It is hoped, however, that the layman will find accuracy sufficient for his needs, and the specialist will be referred to technical literature where he can satisfy his needs for greater detail.

While every effort has been made to ensure accuracy, it is inevitable that errors have crept in. Please call them to our attention.

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H.W. Jackson
Chief Biologist
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CLASSIFICATION - FINDER
for
NAMES OF AQUATIC ORGANISMS
in
WATER SUPPLIES AND POLLUTED WATERS

Part I. The System of Classification

I INTRODUCTION

A Every type of living creature has a favorite place to live. There are few major groups that are either exclusively terrestrial or aquatic. The following remarks will therefore apply in large measure to both, but primary attention will be directed to aquatic types.

B One of the first questions usually posed about an organism is: "What is it?", usually meaning "What is it's name?". The naming or classification of biological organisms is a science in itself (taxonomy). Some of the principles involved need to be understood by anyone working with organisms however.

1 Names are the "key number", "code designation", or "file references" which we must have to find information about an unknown organism.

2 Why are they so long and why must they be in Latin and Greek? File references in large systems have to be long in order to designate the many divisions and subdivisions. There are over a million and a half items (or species) included in the system of biological nomenclature (very few libraries have a million books).

3 The system of biological nomenclature is regulated by international congresses.

a It is based on a system of groups and super groups, of which the foundation (which actually exists in nature) is the species. Everything else has been devised by man and is subject to change and revision as man's knowledge and understanding increase.

b The basic categories employed are as follows:

(1) Similar species are grouped into genera (genus)

(2) Similar genera are grouped into families

(3) Similar familes are grouped into orders

(4) Similar orders are grouped into classes

(5) Similar classes are grouped into phyla (phylum)

(6) Similar phyla are grouped into kingdoms

4 The scientific name of an organism is its genus name plus its species name. This is analogous to our system of surnames (family names) and given names (Christian names).

a The generic (genus) name is always capitalized and the species name written with a small letter. They should also be underlined or printed in italics when used in a technical sense. For example:

Homo sapiens - modern man

Homo neanderthalis - neanderthal man

Esox niger - Chain pickerel

Esox lucius - northern pike

Esox masquinongy - muskellunge

b Common names do not exist for most of the smaller and less familiar organisms. For example, if we wish to refer to members of the

RELATIONSHIPS BETWEEN LIVING ORGANISMS

ENERGY FLOWS FROM LEFT TO RIGHT, GENERAL EVOLUTIONARY SEQUENCE IS UPWARD

PLANTS 5	**ANIMALS 61**		**FUNGI 250**
ORGANIC MATERIAL PRODUCED, USUALLY BY PHOTOSYNTHESIS	ORGANIC MATERIAL INGESTED OR CONSUMED DIGESTED INTERNALLY		ORGANIC MATERIAL REDUCED BY EXTRACELLULAR DIGESTION AND INTRACELLULAR METABOLISM TO MINERAL CONDITION
ENERGY STORED	ENERGY RELEASED		ENERGY RELEASED
FLOWERING PLANTS AND GYMNOSPERMS 76	ARACHNIDS 167	MAMMALS 243	BASIDIOMYCETES 266
	INSECTS 154	BIRDS 242	
CLUB MOSSES, FERNS 76	CRUSTACEANS 129	REPTILES 241	ASCOMYCETES 265
	SEGMENTED WORMS 121	AMPHIBIANS 240	
LIVERWORTS, MOSSES 73	MOLLUSCS 172	FISHES 195	HIGHER PHYCOMYCETES 261
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ALGAE 12	WHEEL ANIMALS 116	STARFISH GROUPS 185	MYCETOZOA 91
	ROUNDWORMS 113		
	FLATWORMS 108		
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DEVELOPMENT OF MULTICELLULAR OR COENOCYTIC ORGANISMS

HIGHER PROTISTA

PROTOZOA 82

DIATOMS 38	AMOEBOID PROTOZOA 86	CILLIATED PROTOZOA 92	LOWER PHYCOMYCETES 261
PIGMENTED FLAGELLATES 12	FLAGELLATED PROTOZOA 85	SPOROZOA 98	
	(COLORLESS FLAGELLATES 85	SUCTORIA 97	

DEVELOPMENT OF A NUCLEAR MEMBRANE

LOWER PROTISTA (OR MONERA)

BLUE GREEN ALGAE 7		ACTINOMYCETES 253
PHOTOTROPIC BACTERIA 252		SPIROCHAETES 255
CHEMOTROPIC BACTERIA 252		MYXOBACTERIA 254
		PARASITIC BACTERIA 251 AND VIRUSES
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NOTE: NUMERALS REFER TO PARAGRAPHS IN PARTS 2 AND 3.

W. B. COOKE AND H. W. JACKSON, AFTER WHITTAKER

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genus *Anabaena* (an alga), we *must simply* use the generic name, and:

Anabaena planctonica,

Anabaena constricta, and

Anabaena flos-aquae

are three distinct species which have different significances to water treatment plant operations.

5 A complete list of the various categories to which an organism belongs is known as its "classification". For example, the classification of a type of frog spittle, a common filamentous alga, and a crayfish or crawdad are shown side by side below. Their scientific names are *Spirogyra crassa* and *Cambarus sciotensis*.

a Examples of the classification of an animal and a plant:

(Frog Spittle)		(Crayfish)
Plantae	*Kingdom*	Animalia
Chlorophyta	*Phylum*	Arthropoda
Chlorophyceae	*Class*	Crustacea
Zygnematales	*Order*	Decapoda
Zygnemataceae	*Family*	Palaemonidae
Spirogyra	*Genus*	Cambarus
crassa	*Species*	sciotensis

b These seven basic levels of organization are often not enough for the complete designation of one species among thousands; however, and so additional echelons of terms are provided by grouping the various categories into "super..." groups and subdividing them into "sub..." groups as: Superorder, Order, Suborder, etc. Still other category names such as "tribe", "division", "variety", "race", "section", etc. are used on occasion.

c Additional accuracy is gained by citing the name of the authority who first described a species (and the date) immediately following the species name. Authors are also often cited for genera or other groups.

d A more complete classification of the above crayfish is as follows:

Kingdom Animalia

Phylum Arthropoda

Class Crustacea

Subclass Malacostraca

Order Decapoda

Section Nephropsidea

Family Astacidae

Subfamily Cambarinae

Genus *Cambarus*

Species *sciotensis* Rhoades 1944

e It should be emphasized that since all categories above the species level are essentially human concepts, there is often divergence of opinion in regard to how certain organisms should be grouped. Changes result as knowledge grows.

f The most appropriate or correct name for a given species is also sometimes disputed, and so species *names* too are changed. The *species* itself, as an entity in nature, however, is relatively timeless and so does not change to man's eye.

II THE GENERAL RELATIONSHIPS OF LIVING ORGANISMS

A Living organisms (as contrasted to fossil types) have long been grouped into two kingdoms: Plant Kingdoms and Animal Kingdoms. Modern developments however have made this simple pattern technically untenable. It has become evident that there are as great and fundamental differences between certain other groups and these (two), as there are between the traditional "plant" and "animal". The accompanying chart consequently shows the Fungi as a third kingdom.

B The three groups are essentially defined as follows on the basis of their nutritional mechanisms:

  1 Plantae: photosynthetic; synthesizing their own organic substance from inorganic minerals. Ecologically known as PRODUCERS.

  2 Animalia: ingest and digest solid particles of organic food material. Ecologically known as CONSUMERS.

  3 Fungi: extracellular digestion (enzymes secreted externally). Food material then taken in through cell membrane where it is metabolized and reduced to the mineral condition. Ecologically known as REDUCERS.

C Each of these groups includes simple, single celled representatives, persisting at lower levels on the evolutionary stems of the higher organisms.

  1 These groups span the gaps between the higher kingdoms with a multitude of transitional forms. They are collectively called PROTISTA.

  2 Within the protista, two principle sub-groups can be defined on the basis of relative complexity of structure:

    a The bacteria and blue algae, lacking a nuclear membrane, may be considered as the lower protista or MONERA.

    b The single celled algae and protozoa having a nuclear membrane, are best referred to simply as the higher protista.

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